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THE EFFECTS OF THE CONSTANT CURRENT ON THE HEART.

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THE Frog and Toad were chiefly employed in the following observations. Several experiments however were made with the hearts of Tortoises and Dog Fish. For a supply of the latter the authors are deeply indebted to the kindness of Mr Henry Lee, of the Brighton Aquarium, and to the liberality of the Directors of that Institution.

The current was always applied by means of non-polarisable electrodes. Tracings of the heart's beat were generally taken by means of a very light simple lever, or levers, placed directly on the ventricle, or on the auricle and ventricle. Occasionally the endocardial method was employed, but all the main results were obtained by means of the lever. Undoubtedly the contact of even the lightest lever must be regarded as a stimulus; but this stimulus is at its maximum at the moment of application, and very rapidly sinks to zero. Practically there is no difficulty whatever in eliminating the effects of the lever from those of the currents.

The results of the observations may be naturally arranged according to the part of the heart subjected to the current, and according to the condition of the heart previous to the application of the current.

1. *The lower two-thirds of the ventricle.*

This portion was chosen as an especial object of study, because at the time our observations were begun it was generally admitted that a ventricle deprived of its basal third, on the one hand contained no ganglionic apparatus, and on the other never exhibited any spontaneous rhythmic beat. Since

our observations were made we have read the interesting observations of Merunowicz[1], who has succeeded in obtaining good spontaneous rhythmic pulsations from this moiety of the ventricle. We cannot but regard his results as corroborative of some at least of our own conclusions.

The results which we obtained on subjecting such a portion of the ventricle to a constant current directed longitudinally, that is from base to apex or from apex to base, differed according to the strength of the current employed.

With very weak currents, sometimes no effect at all is produced; sometimes there is seen a beat at the making of the current, or at the breaking, or at both making and breaking, the tissue during the passage of the current remaining perfectly quiescent. The beat thus brought about is in all its features a normal beat.

In very many cases the making beat was distinctly seen to proceed from the kathode and to travel towards the anode, and the breaking beat to proceed from the anode and to travel towards the kathode. This was most clearly seen when the ventricle was bisected longitudinally almost up to the apex, where a bridge of tissue was left, and the limbs of the V-shaped mass extended into almost a straight line and pinned out in that position. Under favourable circumstances the beat was seen to move as a wave from one pole to the other, from the kathode to the anode or from the anode to the kathode, as the case might be. But the experiment did not always succeed, and occasionally, when probably the bridge left was too small, the two portions acted as two independent masses. We may remark in passing that this experiment quite corroborates Engelmann's[2] views on the *physiological* continuity of the whole ventricular tissue, views which we have fully adopted in our paper on the Snail's Heart[3], where we perhaps ought to have called attention to Engelmann's previous remarks on physiological continuity[4] more specially than we did.

Thus far the cardiac tissue seems to differ in no way from ordinary muscular tissue, and the above results, which remain

[1] *Berichte k. Sächs. Gessellschft. d. Wissenschft.* 1875, p. 254.
[2] Pflüger's *Archiv*, XI. p. 465.
[3] *Proc. Roy. Soc.* XXIII. p. 318.
[4] Pflüger's *Archiv*, II. p. 243.

the same after the heart has been treated with urari or with atropin, are merely illustrations of Pflüger's law.

When however stronger currents are employed, distinct rhythmic pulsations are set up on the making of the current, continue during the passage of the current, and cease with its cessation. The beats thus produced are in all their characters like to normal spontaneous beats; indeed are indistinguishable from them. We were able to obtain these beats from any piece of the ventricle, however small.

The frequency and force of the beats depend on the strength of the current in relation to the irritability of the heart. Thus if a current which is just strong enough to produce simply a making and a breaking beat, be slightly increased, the result is that the making beat is followed at a considerable interval by a second beat while the current is going on, and this perhaps by a third or fourth; and the breaking beat fails to make its appearance. As the current is still further increased in strength, the beats become more frequent and at the same time more forcible, until a point is reached at which the maximum of pulsation is obtained, *i.e.* at which the beats are at the same time strongest and most rapid. Beyond this the increase of frequency still goes on, but at the expense of the force of the individual beats. The beats then tend to overlap and so to convert the rhythmic pulsation into an ordinary tetanus; but for this a very strong current is required; and indeed we were never able to bring about a complete tetanus, such as could be fairly compared with the tetanus of an ordinary striated muscle.

Of the rhythmic pulsation thus brought about many varieties presented themselves, varieties which we can only refer, without completely explaining them, to the varying irritability of the heart.

The most common type is that which, following the phraseology of the Leipzig school, we may speak of as consisting of an ascending and descending staircase. In this the making of the current is followed immediately or after a short interval by a feeble beat, this by a stronger one, and so on in an ascending series until a maximum is reached, which after being maintained for a variable time gives place to a

descending series in which the beats diminish in force and lose in frequency. With currents of short duration the descending limb, as might be expected, is frequently absent.

Very frequently the ascending limb is absent, the pulsations starting at a maximum, and declining either immediately or after a variable period; but, sometimes, even with currents lasting the greater part of a minute, no decline at all is seen, the heart continuing to beat uniformly and regularly during the whole time of the passage of the current.

In nearly all cases, and certainly in all cases where the pulsations are maintained to the end of the passage of the current, the distinct breaking beat is conspicuous by its absence. We have however occasionally but very rarely seen the pulsations survive the application of the current, two or three beats making their appearance after the current had been broken.

This power of the constant current thus to provoke a regular rhythmic pulsation in the part of the ventricle normally devoid of any spontaneous beat was observed long ago by Eckhard[1]; and indeed was the subject of a controversy between that physiologist and Heidenhain[2]. The general impression produced by that controversy seems to have been that the rhythmic pulsations caused by the constant current simply formed a particular case of the tetanus observed by Pflüger[3] as the result of the application of the constant current to an ordinary muscle-nerve.

Without denying that the two sets of phenomena may have a common origin in so far as they may be both due (as Pflüger suggests with regard to one) to electrolytic action, we must confess that in their general features the two are most markedly different. The tetanus set up by the application of the constant current to a nerve is a rapid tetanus; by no adjustment of the strength of the current can the rhythm of the contractions be graduated. With certain strengths of current, the tetanus is absent; and as the strength is increased, a tetanus of rapid rhythm suddenly

[1] *Beiträge zur Anat. u. Phys.* Bd. I. 147, also Bd. II. 123.
[2] Müller's *Archiv*, 1858, p. 479.
[3] Virchow's *Archiv*, XIII.

makes its appearance when a certain point is reached. Very striking is the contrast offered by the cardiac muscle under the same constant current. Under favourable circumstances, and by applying the current with great care, a whole series may be obtained ranging, according to the strength of current employed, from the initial simple make- and break-beat, through a rhythm of two or three beats a minute, to one of a beat every two or three seconds, and finally to one every second, or even to a still more rapid rhythm. Nothing like this is ever witnessed in any ordinary muscle. The beats moreover have all the features of normal beats. An observer, however experienced in cardiac experiments, would, on seeing the pulsations, suppose the heart to be beating naturally and spontaneously, if he did not know that the current was being applied. Indeed, the simplest and perhaps the truest mode of stating the facts is to say that the constant current provokes the heart to spontaneous beats.

Nor are these rhythmic contractions witnessed with the constant current alone. As one of us pointed out some time ago[1], when a quiescent lower moiety of the frog's ventricle is submitted to the action of an ordinary interrupted current for some little time, the irregular tetanic contractions which generally speaking first make their appearance, give place to a rhythmic beat, the features of which are in every way normal. On the supposition that the lower two-thirds of the frog's ventricle do not contain any ganglionic structures, these facts seem to point most distinctly to the conclusion that the less differentiated cardiac muscular tissue still retains a power of rhythmic pulsation, which power has been almost entirely lost by those muscles which are more completely under the dominion of the will. We say almost entirely, because in the first place theoretical considerations would lead us to suppose that if the property of rhythmic movement were a fundamental attribute of primitive protoplasm, traces of this property would be visible even in the most differentiated forms, and in the second place, such traces may indubitably be found[2]. But to say that the cardiac

[1] Foster. This *Journal*, Vol. III. p. 400.
[2] See the interesting paper of Mr Romanes which precedes the present

muscular tissue still retains the power of rhythmic pulsation, is very nearly the same thing as saying that the rhythmic pulsation of the heart is a fundamental property of its general muscular tissue and not of any special localized mechanism. Thus although the lower moiety of the ventricle does not under ordinary circumstances exhibit a spontaneous pulsation, its behaviour under the constant current warrants the conclusion that the power of rhythmic pulsation though latent is not absent, and only needs favourable circumstances for its complete manifestation. Those favourable circumstances are provided for by Merunowicz's (*loc. cit.*) mode of experimentation, and he accordingly finds that the lower moiety of the frog's heart beats with a regularity and spontaneity as distinct if not as ready as that of the entire ventricle. In fact, the distinction between the entire ventricle and the moiety is one of degree, not of kind. The entire ventricle will beat under many circumstances; in fact nearly, though not quite, as readily as the ventricle with the auricles attached. The moiety will only beat under certain favourable circumstances, *e. g.* when its fibres are extended by the distension of its cavity, and at the same time supplied with nutrient or invigorating material, or when it is subjected to a constant current of a certain intensity.

Now it may without any risk be affirmed that distinct ganglionic cells, like those which are found in the atrioventricular boundary and elsewhere, are absent from the lower two-thirds of the ventricle. Structures of that size could not be missed by the many acute observers who have searched for them. We may therefore without any fear say that the spontaneous beat of the heart cannot be due to the action of *such ganglionic cells as these.*

Future observers may discover in the lower moiety of the ventricle nervous structures of another order; and in that case it may become necessary to transfer to those structures the attributes which are now monopolized by ordinary gan-

article, and which bears very closely both on this and on many other points. We may perhaps be permitted to point out that, while the rhythmic pulsations observed by Mr Romanes were done away with by urari poisoning, we have not seen any fundamental changes introduced into our results by the exhibition of even large doses of that drug.

glionic cells. For ourselves, we must confess that it seems more in accordance with the tendency of physiological inquiry to suppose that the cardiac muscle-cells (they might be called neuro-muscular cells if there be any advantage in the name) have not yet lost that property of rhythmic movement which is seen to be exercised with varying but progressive regularity in all protoplasm, from that of a bacterium or a vegetable cell upwards.

The fundamental functional homogeneity and at the same time accidental heterogeneity of the cardiac tissue is shewn in the following observations.

When a large piece of the ventricle was employed, for instance the whole of the lower two-thirds, the rhythmic pulsation was always markedly much more easily obtained when the kathode was placed at the base than when the kathode was placed at the tip.

When the piece was small, *e.g.* when the mere tip of the ventricle was employed, rhythmic pulsations were more readily obtained when the kathode was placed at the tip and the anode at the cut surface, than when the current was sent in the contrary direction.

In a piece cut out from any part of the lower two-thirds, rhythmic pulsations were always more readily produced when the current was thrown in one direction than in another; that is, when one part rather than another was made kathodic. By carrying incisions in various directions we could almost at will convert any spot into a point, the kathodization of which appeared more favourable to pulsation than that of other points.

It seems impossible to attribute these results to any arrangement of localized mechanisms, save such a distribution of them as would be coextensive with the muscular tissue itself. Without being able fully to explain the facts, we are inclined to interpret them as being connected with the shape of the ventricle and the consequent unequal distribution of the anodic and kathodic areas.

It is exceedingly probable that the beats are kathodic in character, *i.e.* that they proceed from the kathodic region, and

are connected with the katelectrotonic phase. We have not however been able to prove this satisfactorily. In many cases, as for instance when the piece of ventricle was divided longitudinally and the parts pinned out, the beats could be distinctly seen to proceed from the kathode. In many other cases, on the contrary, the beats seemed to start in the kathodic and anodic region at the same time. In the snail's heart (*loc. cit.* p. 326) the beats which the constant current evoked in an otherwise quiescent ventricle could very clearly be seen to proceed from the kathode and to travel towards the anode; but when the complicated arrangement of the fibres in the twisted tube which forms the frog's ventricle is compared with that of the fibres in the straight tube of the snail's heart, it is easy to understand why the regular progression from the kathode to the anode, which is so obvious in the latter, should be obscured in the former. On the other hand, the beats which are seen during the passage of the current have all the appearances of being repetitions of the initial beat. But this is a kathodic beat, and we may therefore infer that the following beats are kathodic also. If this be so, then we may go a step further, and conclude that the facility with which beats can be evoked by the constant current, will depend on the ease with which the katelectrotonic phase can be made dominant in the tissue under the influence of the current.

Now, in dealing with the effects of the constant current on nerve-fibres and ordinary striated muscular fibres, we have to do with cylinders of uniform diameter (neglecting, as we may do, the extreme ends of the muscular fibres) throughout, and the relative expanse of the katelectrotonic and anelectrotonic areas will depend entirely on the position of the neutral line, and that again on the strength of the current in relation to the irritability of the tissue. As far as we know, the case has never been considered where the tissue forms a physiologically continuous cone, one electrode being applied to the base and the other to the apex. There is no proof that the anelectrotonic and katelectrotonic phases (*and by these terms we mean the physiological and not the merely electrical conditions of the tissue*) are so related to each other that the movement, whatever it may be, in one direction is accompanied by an

equivalent movement in the other direction, the sum of the two remaining null; we are not debarred, by anything that we know, from the supposition that when the kathode is applied broadly to the base of such a cone, the larger portion of the tissue is thrown into the katelectrotonic state, and when the anode is placed at the base, the smaller portion. In the behaviour of the snail's heart, which forms such a cone of physiologically continuous tissue, we thought we found positive indications of an influence of the form of the tissue in this direction; and since Engelmann has shewn that the ventricle of the frog's heart is similarly physiologically continuous, we have some ground for applying a similar hypothesis to that organ also. If we are allowed to do so, then we should say that the lower two-thirds of the ventricle, in spite of the injury caused by the section, still form a cone, so that when the current is applied with the anode at the tip, the larger portion of the tissue is thrown into katelectrotonus, and beats in consequence are more easily evoked than when the kathode is placed at the tip. When, on the contrary, a small piece only of the ventricle is being operated on, the injury at the lines of section, and the directions of the incisions, determine the position in which the electrodes should be placed in order that the katelectrotonic phase should be dominant, and in consequence beats more easily evoked. We throw out this view with great diffidence as a mere suggestion, put forward in the hope that it may provoke inquiry into what, on any hypothesis, seems a singular fact, that the beats should be produced more easily by applying the current in one direction than in another, and that the direction depends on the form of the piece of ventricle or at least on the directions of the incisions made.

We naturally turned our attention to the point whether the neutral boundary could be shifted, and the relative areas of katelectrotonus and anelectrotonus in consequence varied by varying the strength of the current.

According to the views just expressed it might be supposed that, since the neutral boundary in ordinary nerve electrotonus moves from the anode towards the kathode as the strength of the current is increased, the ease with which beats could be

produced would diminish with the increase in the strength of the current. As a matter of fact the beats are more easily produced with the stronger currents; and this fact seems at first sight distinctly to disprove the hypothesis we have put forward. But, in the first place, it must be remembered that the rhythmically pulsating cardiac tissue differs from a nerve-fibre in this, that with the initial beat, and with each subsequent beat, the condition of the tissue is profoundly altered as the very result of the beat. In the second place, we have in the heart's beat to do with two distinct things; the force and extent of the individual beats, and the rapidity with which the beats are repeated. Between these two things there is a relation, and that relation is in large measure an inverse one. (In the ordinary explanations given of rhythmic action, the two are treated of as being absolutely in an inverse relation. When a gas is bubbling through a fluid the large bubbles come slowly: when they follow rapidly they become small. The problems of the heart would be very simple if this inverse relation were an absolute one for it also; it is not so, for we may have within limits rapid strong beats, and infrequent feeble ones; but nevertheless the inverse relationship does exist though obscured by other influences.)

Now, in operating on the ventricle with a constant current, it is seen that an increase in the strength of the current bears much more upon the rapidity of the rhythm than on the force of the individual beats. We have frequently observed that the beats, few in number, called forth by a weak current are individually as strong as those, many in number, which are produced by a stronger current applied for the same time. Nay more, when a certain limit has been passed the beats lose in force while they gain in rapidity, by increase of the current. In the last matter we have to do probably with the effect of one beat upon another in the shape of exhaustion; and indeed the whole of this part of the subject must remain obscure until we know the limits and conditions of the beneficial and of the injurious effects of any given beat on its successor; for these beneficial effects do exist, as shewn by the labours of Bowditch, and the injurious effects are readily shewn in any overtaxed heart.

If we might make a suggestion it would be in the direction that while the force of the beat is most (but not exclusively) dependent on the area of the katelectrotonus, the rapidity of the rhythm is more immediately connected with the intensity of the electrotonic phases. This would at least enable us to understand why, in spite of the diminished katelectrotonic area, the beats increased in rapidity with an increase in the current[1].

The absence of the break-beat when the current has during its passage evoked a series of pulsations, seems to us a point of interest as contrasted with its very regular presence when the current has simply caused a make-beat, and not given rise to any series. When the beats are maintained during the whole time of the passage of the current, the break-beat is almost invariably absent. It is only when the beats have ceased before the shutting off the current, so that a pause of some length is seen between the last beat and the actual breaking of the current, that the break-beat follows upon a series.

Now the break-beat in many cases was most distinctly seen to be, and in all cases probably is, an anodic beat; it proceeds from the anode, and is due to the disappearance of anelectrotonus. May we infer from this that the effect of a beat is to neutralize the (physiological) electrotonic condition, so that after each explosion neither the kathodic region is so katelectrotonic, nor the anodic so anelectrotonic, as immediately before the beat? that during the interval between that and the succeeding beat the heart is occupied in getting up, so to speak, the katelectrotonic and anelectrotonic phases? This would at least enable us to understand why the larger kathodic area is more favourable for the development of beats.

[1] It is impossible to avoid the conviction that the processes concerned in the production of a pulsation spontaneous or otherwise, are capable of being analysed, like the phenomena which belong to an ordinary muscular contraction, into those pertaining to the stimulus wave and those pertaining to the contraction wave. If this be granted, it is more than probable that the two sets of processes would be differently affected by the constant current; and that this difference might explain the results recorded in the text. Our efforts however to lay hold of this difference have been hitherto wholly in vain.

2. *The whole ventricle, when quiescent.*

As is well known, the entire ventricle, from which the auricles have been carefully removed, though still retaining the ganglia situated at the base of the ventricle, frequently remains perfectly quiescent, without offering any spontaneous beats at all.

Such a ventricle when submitted to the action of the constant current behaves exactly as does a ventricle from which the base has been removed. According to the strength of the current in relation to the irritability of the tissue, the effect of the current may be a simple make- and break-beat, a series of infrequent pulsations, or a series of rapid pulsations. The beats are more easily evoked when the kathode is placed at the base than when it is placed at the tip. In fact, we met with no one feature (except a greater irritability, that is, a greater readiness to respond to comparatively weak currents, and a greater endurance, that is, a capability of being submitted to the action of currents for a longer time without losing its activity) which we could point to as distinguishing the ventricle possessing the atrioventricular ganglia from one devoid of those structures. This fact shews very clearly, on the one hand, how entirely subordinate is the influence of the ganglia in question, as far as the intrinsic activities of the ventricle itself are concerned; and, in the second place, how little permanent damage is done to the ventricle, as a whole, by the rough section needed to remove the ganglia.

3. *The whole ventricle beating spontaneously.*

As is well known also, the frog's ventricle will, in the complete absence of auricles and sinus venosus, frequently go on beating spontaneously for a very considerable time.

When such a spontaneously beating ventricle is submitted to the action of the constant current, the effects are by no means so constant as in the two cases we have already discussed.

With weak currents, that is, with one or two Daniell's cells applied through small non-polarisable electrodes of considerable resistance, no effect whatever was visible. Both in respect to

the rapidity of the rhythm and the force and duration of the individual beats, the heart behaved at the making, at the breaking, and during the passage of the current, in a perfectly normal manner.

By stronger currents, such as those supplied by three to six Grove's, the heart was visibly affected, both at the make and break, and during the passage of the current.

The make and break effects were fairly constant, with the exception that they varied according to the phase of the cardiac cycle which was being passed through at the moment when the current was made or broken. Thus, if the current were made during a certain part of the systole of any beat, that beat was followed by one which was at once premature and slight. This in turn was succeeded by an abnormally prolonged diastole ushering in a beat larger than the normal; after which, at least in the cases where the current was of such a strength as only to produce make and break effects, and not to affect the heart to any marked extent during the passage of the current, the pulsations were normal until the break occurred, which if it took place at the same phase of the systole, produced a similar effect to the make. Hence, both at make and break, a normal beat was followed, first by a short diastole and a feeble beat, and then by a long diastole and a strong beat; and in many cases, the long and short diastoles made up together the length of two normal diastoles, and the movements of the lever during the feeble and strong beats were together about equal to the movements of the lever during two normal beats. We do not, however, pretend to say that this compensation was always exact and complete; and it was certainly less exact as regards the force of the beats than as regards the length of the intervals.

When the current was made or broken at certain other phases of the cardiac cycle, the effects we have just described were replaced by others. Thus, sometimes the initial beat was enlarged; sometimes no obvious effect at all was produced. We did not make a sufficient number of observations to work the point out thoroughly, but hope to be able to do so at some future time, since the facts seem not without interest as promising to throw light on the varying conditions of the heart as it passes through its several phases, and to afford a means of

more accurately measuring than has hitherto been done the duration of the latent period of a natural systole[1].

Far less constant than the above effects were those which made their appearance during the passage of the current.

The most frequently recurring effect was, that during the passage of the current the beats were most distinctly lessened, and with the stronger currents almost completely annihilated, without *any marked change of the rhythm.* This we have seen again and again, both when the kathode was placed at the base and when it was placed at the apex.

We sought, by placing two light levers, one near the base and the other near the apex, to gain some insight into the relative movements of different parts of the ventricle; but we found that the varying pressures of the two levers, and a variety of other circumstances, introduced so many sources of error, that we were unable to arrive at any satisfactory conclusion.

We thought that in many cases, especially where moderate currents were used, we had fairly distinct evidence that when the kathode was at the base there was an increase of movement at the base and a diminution of movement at the apex during the beats, and that, on the contrary, when the kathode was at the apex there was an increase of movement at the apex and a diminution at the base; there being in both cases a diminution in the movements of a lever placed midway between base and apex. But our results were not sufficiently constant to enable us to lay stress on this, which would point to a tolerably satisfactory explanation of the total lessening effect of the current in whatever way applied. We may add, that the same lessening was seen when the current was applied transversely or obliquely instead of longitudinally.

We also thought we noticed that there was a tendency to a quickening of the rhythm, though never very pronounced, when the base was kathodic, and inversely a tendency to retardation when the apex was kathodic; but on this point again we

[1] Since the above observations were made M. Marey (*Comptes Rendus*, 1876) has published a note in which he describes briefly very similar phenomena, and promises to deal with them in fuller detail than we have been able to do.

cannot, in face of the inconstancy of our results, assert anything distinct or certain.

Nevertheless there remains the striking fact that, taking the ventricle as a whole, its spontaneous pulsations are diminished by the passage of a constant current of sufficient intensity. So that between a quiescent ventricle and one which is beating spontaneously, there is this marked contrast in their behaviour under the constant current, that whereas the current evokes pulsations in the quiescent ventricle, it stops, or goes far to stop, the pulsations of the pulsating one. Thus the same current acting on the same ventricle, with the electrodes exactly in the same position, may at one moment all but stop pulsations, and a short time afterwards, when the ventricle has ceased to beat spontaneously, call forth pulsations which are in every way like to the pulsations it just before had stopped.

Both these effects were seen not only in a natural ventricle, but in one which previous to excision had been treated with urari or with atropin to a sufficient extent to do away with the inhibitory action of the vagus. And since the exciting effect on the quiescent ventricle has been shewn to be independent of the action of ordinary ganglia, we may fairly infer that the restraining influence on the pulsating ventricle has likewise but little to do with ganglia, the effects of both kinds being due to the direct action of the current on the cardiac tissue.

4. *The ventricle and auricles, removed from the body and beating spontaneously, with the cavities empty.*

The heart after excision was placed on a block of paraffin scooped out slightly so that the heart remained in one position. The electrodes were placed one at the apex of the ventricle and the other at the sinus venosus or at the upper border of the auricles by the side of the bulbus. Sometimes the heart was placed with the anterior surface uppermost, sometimes undermost. No essential difference was observed in its behaviour in the two different positions.

As far as the ventricle was concerned (and we paid no particular attention to the auricle, the movements of which it is extremely difficult to record satisfactorily) the effect of

the current was exactly the same as when the spontaneously pulsating ventricle was alone operated on. There were the same break and make effects, and the same diminution of the beats during the passage of tolerably strong currents. The effects were essentially the same when urari or when atropin was given, as without those drugs; in fact we failed to distinguish any effects which could be attributed to the inhibitory mechanisms. It seemed as if the influence which the current exercised over the general cardiac tissue overcame altogether any effect which might be produced on the purely nervous structures, and hence ventricle and auricle acted as two organs physiologically isolated, the auricle serving only as a simple conductor of the current to the ventricle, modifying it only by offering resistance to its passage, but otherwise having no effect; and *vice versa.* In this point the frog's heart corresponded entirely with the snail's heart, in which we had[1] previously noticed the same physiological independence.

5. *The ventricle and auricles removed from the body and beating spontaneously, but with the cavities distended with serum.*

We commenced some observations with the current applied to the heart, fitted up for registering the endocardial pressure according to the method of Coates or of Bowditch. The heart was supplied with rabbit's serum or with rabbit's blood diluted with a ·75 per cent. solution of sodium chloride, and the electrodes were applied as usual. We found, however, that the application of the current produced at once such a profound and lasting change in the rhythm, the beats falling into Luciani groups immediately after the passage of even a comparatively weak current, and the groups developing themselves with such vigour, that all further observations were rendered impossible. We have rarely seen an intermittent rhythm so markedly shewn as it was under these circumstances.

[1] *l. c.* p. 325.

6. *The whole heart remaining in the body and the circulation maintained intact.*

The animal was sometimes pithed, but more frequently placed under urari. The heart was sometimes left in its natural position, but sometimes a ligature was thrown round the connective-tissue band which passes from the posterior surface of the ventricle to the adjacent pericardial wall, and the heart turned over, so that the apex pointed to the head, and fixed in that position by the ligature. Notwithstanding this unusual position the circulation went on very well; the advantage of the manœuvre lay in the fact that the levers could be more satisfactorily placed on the ventricle, and the electrodes applied to any part of the sinus venosus. One electrode was placed against the apex, and the other either at the upper border of the auricles or at the sinus venosus, or, in order to eliminate the auricle, at the auriculo-ventricular groove.

The main result which we obtained by applying the current under these circumstances was one which seemed to us very striking. Though we employed tolerably strong currents, *ex. gr.* six Grove cells, and as many as twenty-five Leclanché cells, we could produce no other distinct effects than a making and breaking one.

At the make and break we witnessed very frequently, as in 3 and 4, a premature feeble beat followed by a long pause and a strong beat, when the current was thrown or shut off at the appropriate time; but during the passage of the current itself the pulsations of the heart were in no obvious manner different from the normal.

There was perhaps a general tendency for the beats to be increased in force when the kathode was placed at the auricles or at the base of the ventricle, and a similar tendency for the beats to be diminished when the kathode was at the apex of the ventricle; but this was by no means present with sufficient distinctness and certainty to enable us to say that it was a definite effect of the current.

We applied the current again and again, and for several seconds at a time, without producing any other effects. It was

only after the lapse of several hours, during which the current had been repeatedly applied, that an intermittence in the beat giving rise to irregular groups made its appearance during and for some time after the application of the current.

When one considers how profound are the effects which a constant current of much less strength than that supplied by six Grove cells produces when applied directly to a nerve, it certainly does seem surprising that the heart should be so little influenced by the constant current. The behaviour towards the constant current of the heart supplied under normal conditions with its proper nutritive fluid, when compared with the behaviour of the same heart, either deprived altogether of blood, or fed with serum only, indicates that the apparent indifference of the former is the result of recuperative influences exercised by the blood-supply, and absent in the case of the latter. Some share in the difference between the two might be referred to the isolation of the excised heart placed on the paraffin block, nearly the whole of the current under these circumstances passing into the heart, whereas when the current is applied to the heart in the body, some of it may escape into the surrounding tissues; but this share can only be a very slight one. The real cause of the difference lies in the fact, that the heart which enjoys a rich and continuous blood-supply can accommodate itself rapidly to the new circumstances in which it is placed by the passage of the current, while the nutrition of the heart without a blood-supply is too slow and too feeble to enable it to do so. That the current did produce an effect during the whole time of its passage, (though its action was at a maximum soon after the make), was shewn by the effect at breaking. During the whole of this time katelectrotonic and anelectrotonic phases were established in the ventricle, otherwise the occurrence of the breaking phenomena would be unintelligible. Yet in spite of this the heart continued to beat during the passage of the current at a rate and with a vigour which careful measurements shewed to differ but very slightly indeed, if at all, from the rate and the vigour which obtained previous to the application of the current. Even in the cases where a distinct break effect was absent (and the absence or character, when present, of the break

effect seemed to depend chiefly on the exact phase of the cardiac cycle in which the heart was engaged at the moment when the current was broken), it would be unreasonable to suppose that the current was without effect or had ceased to have any effect; for it can hardly be imagined that the well-nourished and therefore more susceptible heart would be less affected by the current than the ill-nourished and therefore less susceptible heart. It is surely far more in consonance with all the facts to believe that, as we suggested above, the conditions which we know as katelectrotonus and anelectrotonus are developed in connection with spontaneous pulsations, so that, whenever a constant current is applied a struggle takes place between so to speak the natural and the artificial electrotonic conditions, resulting in a defeat of the heart when the heart is weak and the current strong, and in an apparent neglect of the current when the heart is sufficiently active. In this sense we could not speak of any permanent electrotonic condition lasting during the whole time of the passage of the current; since the intensity of the katelectrotonic and anelectrotonic changes would vary during the phases of each cardiac cycle, and thus develope or not a breaking effect, according to the moment at which the current was broken.

An idea presented itself, but only to be rejected, that the pulsations which occurred during the passage of the current were not real spontaneous beats, but artificial beats simulating true ones produced by a current which was strong enough at the same time to place *hors de combat* the ordinary automatic nervous mechanisms. This idea was negatived not only by the fact that the rhythm did not vary with the strength of the current, but also, and more distinctly so, by the fact that, as we subsequently found, the nervous (inhibitory) mechanisms were able to produce, when stimulated, their usual effects in spite of the presence of a strong current.

7. *The ventricles and the auricles brought to a standstill by Stannius' experiment (section of the boundary between auricles and sinus venosus).*

When the heart of the frog is brought to a standstill by this operation, any stimulus applied to the ventricle gives rise

to a beat in which the ventricular systole occurs before the auricular. A series of beats in which this reverse rhythm is manifested, the auricle in each case contracting regularly after instead of before the ventricle, may follow upon the application of a single stimulus. This remarkable feature of the Stannius' standstill was observed by that acute observer Von Bezold[1] (whose early death physiology has so often to deplore), but apparently has not distinctly attracted the notice of subsequent investigators.

Bernstein[2] states that when a constant current is applied lengthways to the heart in this condition rhythmic pulsations (beginning with the making and ending with the breaking of the current) are produced in the direction of the current; *ex. gr.* that the ventricle beats before the auricle when the anode is placed at the apex of the ventricle and the kathode at the auricles, while the auricles beat first when the kathode is placed at the apex and the anode at the auricles.

Bernstein worked with somewhat strong currents, and did not sufficiently vary the strength in different experiments. We find that the result is in close dependence on the strength of the current; and in a series of experiments in which the strength of the current was progressively increased we obtained the following effects.

The animal was generally poisoned with urari in order to eliminate the effects of stimulation of the vagus due to the section from the direct effects of the Stannius' operation; the section was made through the junction of the sinus venosus and auricles; the heart was laid on a paraffin block, and when by its perfect quiescence the operation was seen to have been successful, the current was applied by means of non-polarisable electrodes.

With the weakest currents no effect at all was produced. With somewhat stronger currents rhythmic pulsations were set up on making the current, continued for a shorter or longer time during the passage of the current, and as a rule ceased on the breaking of the current. Both when the base was kathodic and when the apex was kathodic, the beat of

[1] *Physiol. d. Herzbewegung.* Virchow's *Archiv*, XIV.
[2] *Nerv und Muskel*, Abschnitt. v. s. 205.

the auricle succeeded instead of preceding the beat of the ventricle.

When still stronger currents were applied the difference between the case where the apex was kathodic and the case where it was anodic became evident. When the apex was anodic, rhythmic pulsations proceeding continuously from the ventricle to the auricle were set up, the rhythm being more rapid than with the weaker currents. The beats continued during the whole time of the passage of the current. When the apex was kathodic the beats were at first in the order ventricle-auricle; then came a pause of variable duration, after which rhythmic pulsations reappeared, but with *the auricles beating before the ventricle.*

With still stronger currents the events when the apex was anodic remained as before. When however the apex was kathodic the reversal of the order of rhythm took place very early, so that after one or two beats the order ventricle-auricle was replaced by the order auricle-ventricle.

The following details of a series of experiments will perhaps put the facts in a clearer light.

Rana esculenta; urari given. Stannius' experiment successful; heart perfectly quiescent.

Exp. 1. Current supplied by 1, 3 and 5 Leclanché cells respectively. No effect at all produced by the current applied in either direction for 30 seconds. Heart remains perfectly quiescent during and after the application of the current.

Exp. 2. Current supplied by 7 Leclanché cells. Current applied for 30 secs. V means beat of ventricle only. A, beat of auricles only. VA, beat in which the ventricle precedes the auricle. AV, beat in which the auricle precedes the ventricle. The first column gives in each case the time of each beat measured from the making of the current.

Apex kathodic.	Apex anodic.
After ·5 sec. V.	After 12 secs. VA. Beat of ventricle: very large.
„ 1 „ A.	
„ 4 „ VA.	„ 27 „ VA. „
„ 7 „ VA.	„ 30 „ current broken. (No breaking beat.)
„ 30 „ current broken. (No breaking beat.)	

Exp. 3. Current supplied by 7 Leclanché cells; No. 2 repeated.

Apex kathodic.	Apex anodic.
After ·5 sec. V.	After 1·5 sec. VA.
„ 1 „ A.	„ 10 „ VA.
„ 13 „ VA.	„ 24 „ VA.
„ 25 „ VA.	„ 30 „ break.
„ 30 „ break.	(No breaking beat.)
(No breaking beat.)	

After the experiment with apex kathodic had been made, the heart was lightly touched, once only, with a camel's-hair brush soaked in a ·75 solution of sodium chloride. This was done for the purpose of moistening the surface of the heart. A beat in which the ventricle preceded the auricle immediately took place. This was followed by beats, in which the ventricle similarly preceded the auricle, at intervals of 10, 20, 40, 80, 120 seconds respectively after the application of the brush. The heart then became perfectly quiescent, and the other half of the experiment, *i.e.* with the apex anodic, was proceeded with.

Exp. 4. Current supplied by 10 Leclanché cells.

Apex kathodic.	Apex anodic.
After ·5 sec. VA.	After 5 secs. V.
„ 4 „ VA.	„ 5·5 „ A.
„ 7 „ VA.	„ 16 „ VA.
„ 30 „ VA.	„ 25 „ VA.
	„ 30 „ VA.

In this case it was difficult to say if the last beat in each was a simple breaking beat or not. In the case where the apex was kathodic, the long interval preceding (from 7 to 30 secs.) would seem to shew that the beat was a breaking beat; but where the apex was anodic this is not so clear.

Exp. 5. Current supplied by 15 Leclanché cells.

Apex kathodic.	Apex anodic.
After ·5 sec. VA.	After 1 sec. VA.
„ 3·5 „ VA.	„ 4 „ VA.
„ 13 „ VA.	„ 9 „ VA.
„ 21 „ VA.	„ 13 „ VA.
„ 29·5 „ VA.	„ 18 „ VA.
„ 30 „ break.	„ 22·5 „ VA.
(No breaking beat.)	„ 26 „ VA.
	„ 30 „ break.
	(No breaking beat.)

Exp. 6. Current supplied by 20 Leclanché cells.

Apex kathodic.		Apex anodic.	
After ·3 sec.	VA.	After 1 sec.	VA.
" 3 "	VA.	" 4·5 "	VA.
" 5 "	VA.	" 8 "	VA.
" 7·5 "	VA.	" 11 "	VA.
" 10 "	VA.	" 15 "	VA.
" 13 "	VA.	" 19 "	VA.
		" 21 "	VA.
" 26 "	*AV.*	" 24·5 "	VA.
" 32 "	*AV.*	" 28 "	VA.
		" 30 "	VA.

The last beat registered with the apex anodic was not a strictly breaking beat; the contraction began before the current was actually shut off.

In the case where the apex was kathodic the current was kept on two seconds beyond the half minute, being broken immediately after the commencement of the last beat registered.

Exp. 7. Current supplied by 20 Leclanché cells.

Apex kathodic.		Apex anodic.	
After ·2 sec.	VA.	After 2 secs.	VA.
" 6 "	VA.	" 3 "	VA.
		" 6·5 "	VA.
" 10 "	AV.	" 10 "	VA.
" 14 "	AV.	" 13 "	VA.
" 16 "	AV.	" 17 "	VA.
" 19 "	AV.	" 19 "	VA.
" 22 "	AV.	" 22 "	VA.
" 25 "	AV.	" 24·5 "	VA.
" 28 "	AV.	" 28·5 "	VA.
" 30 "	AV.	" 30 "	VA.

In the case where the apex was anodic, at the beginning and at the end of the series a distinct interval was visible at each beat between the contraction of the ventricle and that of the auricles; in the middle of the series, on the other hand, the beat of the auricles came so rapidly after that of the ventricle that they appeared almost synchronous, and it became very difficult to say that the ventricle did really precede the auricles.

In this and several preceding experiments, the beat of the ventricle when it preceded that of the auricles was seen to be preceded in turn by a beat of the bulbus arteriosus.

It will be seen from the above, which is one of many experiments having exactly the same general features, that our

results in large measure agree with those of Bernstein[1], though they differ to such an extent as to prevent our accepting the interpretation given by that inquirer.

That interpretation, if we understand it aright, is as follows. When the apex is made anodic,—when therefore the current may in relation to the heart be said to be ascending,—the nerves which descend from the atrio-ventricular ganglion to the ventricle, are at their origin from the ganglion thrown into katelectrotonus. This weakens the development of the molecular inhibitory processes in these nerves at their origin from the ganglion, and thus favours the development of a beat. The ventricle, thus assisted, in consequence beats before the auricle. When, on the other hand, the apex is made kathodic, and thus the current descending, the same ventricular nerves are thrown into anelectrotonus, which favours the molecular inhibitory processes. The ventricle thus hampered beats after the auricle. Further, while the nerves descending to the ventricle are thus being affected by the respective currents, the nerves ascending from the ganglion to the auricles are being affected in exactly the converse manner; so that while inhibition is being augmented in the ventricle, it is being decreased in the auricle, and *vice versâ*. Hence the dependence of the sequence of the rhythm on the direction of the current.

Now, in the first place, this view entirely overlooks the important fact that in the remarkable condition brought about by Stannius' operation the sequence of auricle upon the ventricle is the normal order of the rhythm of the beat. One instance of this has been mentioned in the foregoing experiment (3); and it will be observed that the stimulus, itself of the slightest character, was followed not by one but by a series of beats, in each of which the contraction of the auricle followed that of the ventricle. Many more instances of the same kind might be given. No great stress could be laid on a single beat with this abnormal sequence making its appearance; but the fact that a whole series having the same character should be regularly carried on, after being started only by the very slightest stimu-

[1] We failed altogether to observe the making and breaking "simultaneous contractions of all parts of the heart" of which Bernstein speaks. This is probably to be explained by the fact that our currents were in general weaker than those used by him.

lus, shews that the heart must, under the circumstances, be in a peculiar condition. No such change of the order of rhythm is witnessed in ordinary pneumogastric inhibition; and there are many reasons, to which we shall presently add a new one, for concluding that the standstill produced by Stannius' operation is fundamentally different in nature from that produced by stimulation of the pneumogastric.

Hence what needs to be explained is not so much why with the apex anodic (or current ascending) the ventricle-auricle order of rhythm is maintained, as why with the apex kathodic (or current descending) this natural order of rhythm is exchanged for that of auricle-ventricle, which in a heart during a Stannius' standstill is an abnormal rhythm.

In the second place, the reversal of the order is only obtained with comparatively strong currents, and in none of the cases we have had under our notice did it occur on the making of the current, being always preceded by one or more beats in which the order was ventricle-auricle, though we are not prepared to say that with still stronger currents than those we used the reversal might not coincide with the beginning of the application of the current. Bernstein[1] seems to have observed this reversal, but in the opposite sense, and he does not appear to have paid much attention to it. His explanation moreover fails to explain why a current of moderate intensity should produce a reversal in the course of or towards the end of its action; see *antea*, Exp. 6.

No solution of the phenomena can be considered satisfactory which is not at the same time a solution, or an approximation towards a solution, of the difficult problem, why in a normal heart-beat the sequence of the constituent contractions is always such as it is, even in a heart whose cavities are empty, and in which therefore the filling or distension of one cavity by the contraction of another can have no share in the matter. That the sequence is not the result of any fixed molecular constitution of the ganglia is shewn by the very fact of the possibility of its reversal. That the sequence may be changed by circumstances indicates that its normal character is due to a concurrence of circumstances, which concurrence is more readily

[1] *l. c.* p. 223.

brought about than any other arrangement. If we suppose the several parts of the heart, ventricle, auricle, sinus venosus, &c., to be mere passive instruments receiving stimuli or impulses to contraction from some common automatic ganglion (situate in the sinus venosus or elsewhere),—the sequence of the impulses being determined by molecular changes in that ganglion and in that alone,—then changes taking place in the ventricle can only affect the extent and character of its own contraction, and not in any way the sequence of the rhythm; and so with other parts. In the experiments above recorded a definite sequence of one kind or of the other was observed in the entire absence of the sinus venosus. On the above view they must be produced by a ganglion or ganglia situate in the auricles. We must further admit that this ganglion is the seat of the normal sequence in rhythm of the entire heart, or suppose that in the absence of the sinus venosus a ganglion, hitherto having no share in the direction of *the sequence*, comes into play. All of which is very complicated and unsatisfactory.

On the other hand, we have the undoubted fact that the ventricle alone (or even part of the ventricle), the auricles alone, the sinus venosus alone, and the bulbus arteriosus alone, can carry on each by itself a rhythmic pulsation of long duration and wholly like that of the entire heart. This means that each of these several parts of the heart has a rhythm of its own dependent on its own circumstances, including under circumstance everything which affects the nutrition both of its muscular and nervous elements. Now when ventricle and auricle are separated from each other, they beat each with an independent rhythm; but when physiologically connected, they beat in harmony and in sequence. It is impossible to conceive of this harmony being accomplished otherwise than by some mutual action of the two upon each other. We cannot suppose that any event connected with the contraction of the one (such as the negative variation of the natural current) by acting as a stimulus determines the contraction of the other. For, in that case, the systole of the ventricle would provoke a systole of the auricles, as well as the systole of the auricles a systole of the ventricle, and a rhythmic pulsation with long pauses between the whole beat (of both auricles and ventricle) would be impos-

sible. Moreover, a weakly ventricle yoked to strong auricles would soon be driven to exhaustion, and *vice versâ*. We are thus driven to the conclusion that the beat of either organ is dependent not only on its own circumstances, but also on the circumstances of its fellow; that the rhythm of the auricles, for instance, is dependent not only on their own condition, but also on that of the ventricle. That just as the beat of the ventricle or of a part of the ventricle is determined by the condition of the whole of the ventricle or the whole of the part (the physiological continuity of the tissue permitting each fibre or bundle of fibres to influence all the other fibres by a sort of muscular sense, so that each fibre or bundle of fibres, instead of pulsating in a rhythm of its own, joins all the other fibres, or bundles, in a rhythm which is that of the whole tissue), in the same way the condition of the whole ventricle (the summation of the condition of the several fibres) is able, by the nervous continuity of auricle and ventricle, to affect, and in turn be affected by, the condition of the auricles, which again is the summation of the condition of the several auricular fibres. Were it permitted to speak of feeling in the absence of consciousness, we might say that just as each fibre of the ventricle (or auricle) feels the condition of and exerts in consequence an action on all the other fibres, whereby a harmony of the whole ventricle (or auricle) is established, so the auricles feel the condition of and exert an action on the ventricle, and *vice versâ*, whereby the harmony of the two is maintained; the nervous structures connecting them being the agents of the intercourse, the nerve-fibres probably serving simply as conductors, while in the nerve-cells processes may go on which stand in about the same relation to processes taking place in the more purely muscular elements that arithmetical operations on logarithms do to operations on the corresponding numbers.

If it be permitted to hold some such provisional view as that which we have just attempted to sketch, we should be obliged to add, that in the normal heart the nutrition of all parts of the heart is, so to speak, tuned for the production of a beat with the normal sequence of sinus venosus, auricle, ventricle, and bulbus. And further, that though the rhythm is at bottom dependent on the condition of the whole heart, yet

each cardiac cycle is *set going* by the contraction of the sinus, the pulsation of that part having just the effect necessary to start the already prepared auricle, and this in turn the ventricle. Hence one might readily imagine that after removal of the sinus venosus (putting aside the effects of the section), the heart, having lost so to speak its leader, would be at a loss how to beat. When it did begin to beat we should expect it to beat in the order auricle-ventricle. The facts, however, that in Stannius' experiment the order ventricle-auricle makes its appearance, and that the ventricle separated from the auricles resumes its pulsations, while the auricles remain quiescent, shew that loss of leadership is not the sole cause of the standstill, but that some inhibitory work (not however of the pneumogastric kind) is going on, which inhibition bears more particularly upon the auricles. Both auricle and ventricle are prepared to beat upon a sufficient stimulus, but they are restrained from spontaneous pulsation by the (at present inexplicable[1]) inhibitory influences started by the section (or ligature) of the sinus venosus, the auricle being more restrained than the ventricle. Hence when a slight stimulus is applied to the heart, a beat, in the order ventricle-auricle, is produced; and that beat, as we know from the researches of Bowditch, being beneficial to the heart, and the inhibitory influences still continuing to work more upon the auricle than the ventricle, the initial beat may be followed by many others having the same sequence of ventricle-auricle.

So, with weak constant currents, which may be regarded as slight stimuli, the same kind of pulsation, the same sequence of ventricle-auricle, is produced, whether the current be ascending or descending.

It is well known that the make and break of a constant current is a more powerful stimulus on muscle than an induction-shock, there being in this point a remarkable differ-

[1] We say "at present inexplicable" because the Stannius' standstill has not yet, in spite of all that has been written on it, been fully cleared up. The fact that the standstill takes place when the endings of the vagus fibres have been paralyzed by atropin, proves that the inhibition is not due simply to vagus stimulation. On the other hand the curious results of Pagliani (*Moleschott's Untersuch.* XI. p. 358), shewing that *gradual* separation of the sinus will not produce standstill, disprove the view that the removal of an automatic ganglion in the sinus is the whole cause of the quiescence.

ence between ordinary muscle and ordinary nerve. In the course of our experiments we have been gradually impressed with the view that in applying the constant current to the heart, the effects which are produced (and we cannot help thinking that electrolysis has much more to do with these than is generally admitted) by the action of the current on the more distinctly muscular elements override those which are due to the action of the current on the more distinctly nervous elements. We may indeed go almost so far as to say that the former put the latter altogether on one side. So that in studying the action of the current on the auricles and ventricle, we have been led to consider merely its action on the muscular elements of the ventricle and of the auricles respectively, without paying any attention to either the inhibitory or any other nervous mechanisms present or supposed to be present. We have at least never met with any satisfactory evidence of the excitation of these nervous structures playing any part in the phenomena with which we have had to deal.

Hence in applying the constant current to the auricles and ventricle we have considered only the effects on the muscular tissue of the one and of the other. Now the ventricle, just as it is more muscular, is more susceptible to the action of the current than the auricle. It is more especially in the ventricle, we might say exclusively in the ventricle, that any difference is observable between the effects of the ascending and those of the descending current. In studying the snail's heart we were very much struck with the greater susceptibility of the ventricle as compared with the auricle. The latter is far less readily inhibited by the interrupted current than the former, less easily roused from quiescence into pulsations by the constant current, less easily checked by the constant current when beating spontaneously. Whether these facts are to be explained as mass effects or in some other way, the frog's heart, in spite of the presence of all its nerves and ganglia, acts in these respects very similarly to a snail's heart; and we venture to suggest that this greater susceptibility to extrinsic influences of the ventricle as compared with the auricle has to do both with the inverted order of sequence so characteristic of the heart during the

Stannius' standstill, and with the fact that the same order is also visible in pulsations called forth by weak constant currents.

The reversal which takes place during the action of the descending current is not so easy to explain; and it is with the greatest hesitation that we submit the following suggestions.

In the first place, in the course of many repeated observations, we were struck with the fact that the descending current had distinctly a more *exhausting* effect on the heart than the ascending current. During the minutes which followed upon the application of the descending current for 30 seconds, the heart was less irritable than it was after the application of the ascending current for the same time. So that, unless care were taken to allow sufficient intervals of restorative rest, the primary effects of the action of the current were obscured by the secondary effects of exhaustion. This exhaustion was of course more evident with strong than with weak currents. The fact that the reversal takes place with weaker currents towards the end and with stronger currents towards the beginning of the action of the current, points very distinctly to exhaustion as a prominent factor in its causation.

In the second place, if we may assume, in accordance with Pflüger's results on nerves, that with weak currents the neutral point lies near the anode, then so long as the current is not too strong a large part of the ventricle will always be in the condition of katelectrotonus, so that whether the base or the apex be kathodic, the area of katelectrotonic tissue in the ventricle is sufficient to maintain the greater susceptibility of the ventricle as compared with the auricle. As the current becomes stronger, the neutral line is driven nearer and nearer to the kathode. Under these circumstances, when the current is descending the base becomes largely anodic. This condition of the ventricle, with the base anodic and the apex kathodic, as shewn very distinctly by the phenomena of the snail's heart, and more or less forcibly illustrated by the foregoing observations on the frog's heart, is equivalent to a preponderating anodisation of the entire ventricle, more being lost by the anodic

condition of the broad base than is gained by the kathodic condition of the narrow apex. And this anodic condition added to the exhaustion (of which it is probably the cause) so depresses the ventricle that the auricle gains the upper hand and precedes it in each beat; the depression of the ventricle however not being so intense as to prevent it from following the auricle at each beat.

When, on the other hand, a strong current is ascending, and the base therefore is kathodic, however much the neutral line is driven near to the base, there is always left an area of kathodic tissue at the broader pollent base sufficient just to maintain that preponderance of the ventricle over the auricle which is the characteristic of Stannius' standstill; and exhaustion not being produced so readily in this case as in the other, the rhythm ventricle-auricle is carried on in spite of the peculiar condition of the former.

We repeat that we put forward this explanation with much hesitation, but we submit that it is founded on at least no greater assumptions than that of Bernstein. At any rate our view has a certain value in reducing the phenomena to known actions of the constant current on irritable tissues.

8. *The heart brought to a standstill by stimulation of the vagus.*

So struck were we, in the course of our experiments, with the entire absence of any phenomena, which we could satisfactorily attribute to the action of the constant current on the termination of the vagus fibres, or on the various inhibitory and other mechanisms existing or supposed to exist in the vertebrate heart, the stimulation of which we, on starting our investigations, supposed would render the behaviour of the frog's heart under the current entirely different from that of the nerveless snail's heart, that we were led to suspect that the currents employed being so much stronger than those generally made use of in experimenting on nerves, exhausted on their first application all the nervous elements, and left us dealing with (so to speak) the naked contractile elements.

To have proved that this was the case would have been to bring an additional and strong argument in favour of the thesis of which all our experiments may be regarded as illustrations, that the causes of the rhythmic pulsations of the heart are to be sought for in the properties of contractile tissue. But we found, to our great astonishment, evidence that the most important and active nervous mechanisms of the heart were able fully to exert their influence in spite of a powerful constant current being passed through the heart. Seeing that a portion of the ventricle, or the whole ventricle when quiescent from whatever reason, or the whole heart when quiescent from the experiment of Stannius, is roused into rhythmic pulsations by the constant current, we very naturally expected that the same current would produce rhythmic pulsations when applied to a heart in standstill from stimulation of the vagus.

We found, however, that this was not the case.

Having brought the heart to a standstill by stimulating the pneumogastric with the interrupted current, we threw into the heart constant currents of various strengths, both ascending and descending. But not even with six Grove cells did we succeed in calling forth any rhythmic pulsations. The only effect which we could trace was a very marked reaction, when both the constant current and the vagus stimulation were removed, the heart soon beginning to beat with remarkable vigour and rapidity.

Having applied the constant current, both in the descending and ascending direction, we stimulated the vagus while the current was still passing through the heart. Inhibition was nevertheless produced, and, as far as we could see, took place very much as if no current were passing through the heart. We are not prepared to say that the current made absolutely no difference or that it would not be possible to call forth rhythmic pulsations in pneumogastric inhibition by applying still stronger currents; but we do say that currents distinctly stronger than those which readily rouse into rhythmic pulsations the naturally quiescent ventricle or part of the ventricle, or the whole heart brought to a standstill by Stannius' experiment, failed with us to bring forth pulsations in a heart brought to a standstill by stimulation of the vagus.

From this we draw the following conclusion. We have argued that the pulsations which the constant current calls forth in a piece of the ventricle must, until some hitherto unnoticed nervous elements have been discovered, be considered as pulsations caused by the direct action of the current on the cardiac muscular tissue. Since these pulsations are also seen in the whole ventricle (otherwise quiescent), and indeed in the whole heart (under Stannius' experiment), when the constant current is applied, it is clear that previous division of the ventricle is not necessary to their production, that the continuity of the ganglionless apex of the heart with those portions of the heart which do contain ganglia, is no bar to pulsations arising in the former as the result of the application of the constant current. Now, in the generally accepted theory of inhibition, the action of the pneumogastric is supposed to stop at certain ganglionic centres. In these centres the impulses descending the vagus so exalt, either directly or by the mediation of various mechanisms, the molecular inhibitory forces that the accustomed rhythmic stimuli are no longer set free, and the muscular fibres lie idle till the struggle in the ganglia is over. According to this view then, whether the muscular fibres are removed from the influence of the ganglia by section or by profound urari or other poisoning, or by the ganglia being preoccupied in an inhibitory struggle, the constant current acting directly on the fibre ought in all three cases to produce the same effect, viz. a rhythmic pulsation. As a matter of fact, it does so in the two former cases but not in the third. From this it follows that stimulation of the vagus, in addition to whatever effect it may have on the ganglia, has also an effect of such a kind that the irritability of the cardiac muscular tissue itself is impaired, and the production of rhythmic pulsations hampered in their muscular origin. The depression of irritability thus caused is not so great but that a mechanical stimulus will produce a contraction, and hence the heart, in standstill from stimulation of the vagus, will beat when pricked. Such a method of estimating irritability is however a very rough one. That a mechanical stimulus calls forth a beat, proves that the irritability is not extinguished, but is no evidence that it is not impaired: the more delicate test of the constant current manifests

the muscular weakness which stimulation of the vagus has caused.

We are thus led to the conclusion that the pneumogastric, like any other motor nerve, acts when stimulated directly on the muscular tissue with which it is connected; from which conclusion there follows, as a corollary, the view that the peculiar inhibitory effects of stimulation of the pneumogastric are due, not to a specific energy of the nerve itself or of any mechanism in which it terminates, but to the fact that while ordinary nerves are connected with muscles ordinarily at rest, the pneumogastric is connected with a muscle in a state of continued rhythmic pulsation. To which we may add, that the other marked inhibitory nerves, the vaso-dilator nerves, are also in connection with muscles normally in a state of activity (tonic action) which is more closely allied to rhythmic pulsation than to any other form of muscular activity; indeed, in many cases, as in the rabbit's ear, the two merge into each other, and it seems difficult to regard the tonic contraction of blood-vessel, in any other light than that of an obscure rhythmic pulsation.

If this view be accepted, the phenomenon of inhibition of the snail's heart by direct application of an interrupted current ceases to be extraordinary; for both that form of inhibition, and the ordinary pneumogastric inhibition, fall into the same category, being both at bottom due to the fact that in them stimulation is brought to bear on a spontaneously active tissue. Against the identity of the two, there may be urged two strong objections, one that the interrupted current applied directly to the vertebrate heart never produces a distinct inhibition similar to that seen in the snail's heart, but a tumultuous irregular sort of tetanus (never however reaching the distinct form of tetanus, and eventually giving rise to a standstill), and the other that stimulation of the pneumogastric never causes (however strong the current) a tetanic contraction of the heart, such as is seen when a too strong interrupted current is applied directly to the snail's heart. Without prolonging the discussion any further, we may be permitted to say that these objections do not seem to us insuperable, and that the study of the vaso-dilator and vaso-constrictor nerves appears likely to afford a solution of the difficulties.

The conclusions then to which our observations point, we do not pretend to say satisfactorily establish, are as follows.

The vertebrate heart, such as that of the frog, behaves towards the constant current in a manner very closely resembling that in which the snail's heart behaves.

The well known, easily recognised, ganglia of the heart play a subordinate part in the production of the heart's spontaneous rhythmic pulsations. The real origin of these is to be sought for in the phenomena of muscular tissue, unless some new form of nervous tissue which has hitherto escaped detection be discovered.

The constant current may according to circumstances call forth or put an end to rhythmic pulsations: calling them forth when they are absent and diminishing or destroying them when they are spontaneously present. Hence, here, as in the case of the snail's heart, stimulation and inhibition are shewn to differ from each other in degree, or according to circumstance, rather than in kind.

Stimulation of the vagus produces an effect on the muscular tissue of the heart; its inhibitory action is not confined to the ganglia; and hence vagus inhibition does not differ so essentially from the inhibition of the snail's heart by direct stimulation as might at first appear.

POSTSCRIPT.

WHILE the above paper was in the printer's hands we received the *Centralblatt f. med. Wissenschaft*, of May 27th (No. 22, 1876), containing a brief communication from Prof. Bernstein *Ueber den Sitz der automatischen Erregung im Froschherzen.*

In it the author relates an experiment in which the ventricle of a frog's heart is violently compressed for a few seconds across its middle with a fine pair of forceps. The line of tissue thus injured breaks the physiological continuity between the upper and lower half of the ventricle; though, there being no actual physical solution of continuity, the apex is still as before supplied with abundance of fresh blood. Since under these circum-

stances the apex, though irritable towards stimuli, remains perfectly quiescent, never exhibiting any spontaneous pulsations, Bernstein argues that the pulsations witnessed in Merunovicz's experiment are not really automatic, but the result of the rabbit's blood or serum acting as a stimulus "upon certain motor mechanisms in the cardiac muscles, and thus causing in them an intermittent discharge of energy."

Without waiting for the results of the counter experiments which naturally suggest themselves, we should like at once to remark that, if a stimulus so constant in its nature and action as serum or blood must be is capable of producing rhythmic movements so varied in their rate of development and character as those which made their appearance in Merunovicz's experiments, the hypothesis of an automatic centre confined to the sinus venosus needs a fresh definition.

The old view, and the one against which we have in foregoing pages argued, taught that the impulses which caused the heart's beat proceeded *in a rhythmic manner* from the ganglia in the sinus, that the rate and character of the rhythm was determined there, and that the muscular apparatus of the heart had no other task than to respond to those rhythmic impulses according to the measure of its irritability. If however, in accordance with Bernstein's new view, the muscular tissue of the heart with its "motor mechanisms" is capable, when affected by a constant stimulus (whether chemical, as blood and sodium chloride, or electrical, as the constant current), of developing rhythmic pulsations *which in no way, except as far as relates to their causation, differ from normal spontaneous beats,* the need of any intermittence in the action of the automatic ganglia in the sinus is done away with; its presence would shew a wasteful want of economy.

Looking at the matter from an evolution point of view, and seeing that muscular or neuro-muscular tissue is anterior in evolution to strictly differentiated nervous tissue, it is in the highest degree improbable that, supposing the power of generating automatic rhythmic impulses were at some time transferred from undifferentiated protoplasmic or neuro-muscular tissue to purely nervous mechanisms, the muscular remnant, from which spontaneity had been removed, would retain a

power of intermittent action which it could never, in actual life, have an opportunity of manifesting, since its intermittence would ever afterwards be determined by its nervous master.

It becomes necessary, therefore, to modify the hypothesis of an automatic centre in the sinus in the sense that the action of that centre is not an intermittent but a continuous one. From this modified view to the one which we ourselves have urged in the foregoing pages, the step is very slight.

We may add that the 20th number of the same *Central-blatt* contains an original communication from Herr Fischer which, while confirming the presence of a considerable number of fine nervous plexuses between the fibres in the ventricle of the dog's heart, throws much doubt on the nervous character of the elements described by Gerlach, as abounding in the striated muscle and in the tissue of the frog's ventricle, elements which might be regarded by some as the long sought for motor mechanisms.

From the *Journ. of Anat. and Phys.*, Vol. X.]

ON THE SPINAL NERVES OF AMPHIOXUS.—By F. M. BALFOUR, B.A., *Fellow of Trinity College, Cambridge.*

DURING a short visit to Naples in January last, I was enabled through the kindness of Dr Dohrn, to make some observations on the spinal nerves of Amphioxus. These were commenced solely with the view of confirming the statements of Stieda on the anatomy of the spinal nerves, which, if correct, appeared to me to be of interest in connection with the observations I had made that, in Elasmobranchs, the anterior and posterior roots arise alternately and not in the same vertical plane. I have been led to conclusions on many points entirely opposed to those of Stieda, but, before recording these, I shall proceed briefly to state his results, and to examine how far they have been corroborated by subsequent observers.

Stieda[1], from an examination of sections and isolated spinal cords, has been led to the conclusion that, in Amphioxus, the nerves of the opposite sides arise alternately, except in the most anterior part of the body, where they arise opposite each other. He also states that the nerves of the same side issue alternately from the dorsal and ventral corners of the spinal cord. He regards two of these roots (dorsal and ventral) on the same side as together equivalent to a single spinal nerve of higher vertebrates formed by the coalescence of a dorsal and ventral root.

Langerhans[2] apparently agrees with Stieda as to the facts about the alternation of dorsal and ventral roots, but differs from him as to the conclusions to be drawn from those facts. He does not, for two reasons, believe that two nerves of Amphioxus can be equivalent to a single nerve in higher vertebrates: (1) Because he finds no connecting branch between two succeeding nerves, and no trace of an anastomosis. (2) Because he finds that each nerve in Amphioxus supplies a complete myotome, and he considers it inadmissible to regard the nerves,

[1] *Mém. Acad. Pétersbourg*, Vol. XIX.
[2] *Archiv f. mikr. Anatomie*, Vol XII.

which in Amphioxus together supply *two myotomes*, as equivalent to those which in higher vertebrates supply *a single myotome only.*

Although the agreement as to facts between Langerhans and Stieda is apparently a complete one, yet a critical examination of the statements of these two authors proves that their results, on one important point at least, are absolutely contradictory. Stieda, Pl. III. fig. 19, represents a longitudinal and horizontal section through the spinal cord which exhibits the nerves arising alternately on the two sides, and represents each myotome supplied by *one nerve.* In his explanation of the figure he expressly states that the nerves of one plane only (*i.e.* only those with dorsal or only those with ventral roots) are represented; so that if all the nerves which issue from the spinal cord had been represented double the number figured must have been present. But since each myotome is supplied by *one* nerve in the figure, if all the nerves present were represented, each myotome would be supplied by two nerves.

Since Langerhans most emphatically states that only *one nerve* is present for *each myotome,* it necessarily follows that he or Stieda has made an important error; and it is not too much to say that this error is more than sufficient to counterbalance the value of Langerhans' evidence as a confirmation of Stieda's statements.

I commenced my investigations by completely isolating the nervous system of Amphioxus by maceration in nitric acid according to the method recommended by Langerhans[1]. On examining specimens so obtained it appeared that, for the greater length of the cord, the nerves arose alternately on the two sides, as was first stated by Owsjannikow, and subsequently by Stieda and Langerhans; but to my surprise not a trace could be seen of a difference of level in the origin of the nerves of the same side.

The more carefully the specimens were examined from all points of view, the more certainly was the conclusion forced upon me, that nerves issuing from the ventral corner of the spinal cord, as described by Stieda, had no existence.

Not satisfied by this examination, I also tested the point by

[1] *Loc. cit.*

means of sections. I carefully made transverse sections of a successfully hardened Amphioxus, through the whole length of the body. There was no difficulty in seeing the dorsal roots in every third section or so, but not a trace of a ventral root was to be seen. There can, I think, be no doubt, that, had ventral roots been present, they must, in some cases at least, have been visible in my sections.

In dealing with questions of this kind it is no doubt difficult to prove a negative; but, since the two methods of investigation employed by me both lead to the same result, I am able to state with considerable confidence that my observations lend no support to the view that the alternate spinal nerves of Amphioxus have their roots attached to the ventral corner of the spinal cord.

How a mistake on this point arose it is not easy to say. All who have worked with Amphioxus must be aware how difficult it is to conserve the animal in a satisfactory state for making sections. The spinal cord, especially, is apt to be distorted in shape, and one of its ventral corners is frequently produced into a horn-like projection terminating in close contact with the sheath. In such cases the connective tissue fibres of the sheath frequently present the appearance of a nerve-like prolongation of the cord; and for such they might be mistaken if the sections were examined in a superficial manner. It is not, however, easy to believe that, with well conserved specimens, a mistake could be made on this point by so careful and able an investigator as Stieda, especially considering that the histological structure of the spinal nerves is very different from that of the fibrous prolongations of the sheath of the spinal cord.

It only remains for me to suppose that the specimens which Stieda had at his disposal, were so shrunk as to render the origin of the nerves very difficult to determine.

The arrangement of the nerves of Amphioxus, according to my own observations, is as follows.

The anterior end of the central nervous system presents on its left and dorsal side a small pointed projection, into which is prolonged a diverticulum from the dilated anterior ventricle of the brain. This may perhaps be called the olfactory

nerve, though clearly of a different character to the other nerves. It was first accurately described by Langerhans[1].

Vertically below the olfactory nerve there arise two nerves, which issue at the same level from the ventral side of the anterior extremity of the central nervous system. These form the first pair of nerves, and are the only pair which arise from the ventral portion of the cerebro-spinal cord. The two nerves, which form the second pair, arise also opposite each other but from the dorsal side of the cord. The first and second pair of nerves have both been accurately drawn and described by Langerhans: they, together with the olfactory nerve, can easily be seen in nervous systems which have been isolated by maceration.

In the case of the third pair of nerves, the nerve on the right-hand side is situated not quite opposite but slightly behind that on the left. The right nerve of the fourth pair is situated still more behind the left, and, in the case of the fifth pair, the nerve to the right is situated so far behind the left nerve that it occupies a position half-way between the left nerves of the fifth and sixth pairs. In all succeeding nerves the same arrangement holds good, so that they exactly alternate on two sides.

Such is the arrangement carefully determined by me from one specimen. It is possible that it may not be absolutely constant, but the following general statement almost certainly holds good.

All the nerves of Amphioxus, except the first pair, have their roots inserted in the dorsal part of the cord. In the case of the first two pairs the nerves of the two sides arise opposite each other; in the next few pairs, the nerves on the right-hand side gradually shift backwards: the remaining nerves spring alternately from the two sides of the cord.

For each myotome there is a single nerve, which enters, as in the case of other fishes, the intermuscular septum. This point may easily be determined by means of longitudinal sections, or less easily from an examination of macerated specimens. I agree with Langerhans in denying the existence of ganglia on the roots of the nerves.

[1] *Loc. cit.*

From the *Journ. of Anat. and Phys.*, Vol. XI.]

THE ACTION OF PILOCARPIN ON THE SUB-MAXILLARY GLAND OF THE DOG. By J. N. LANGLEY, B.A., *St John's College, Cambridge.*

IT has for some time been well known that jaborandi causes in man and the higher vertebrates a very marked salivary secretion. It might then not unnaturally be supposed, that an inquiry into its method of action would possibly suggest some more complete explanation than we have at present of the salivary secretion, and so of secretion in general. The following experiments were begun with that end in view; but the experiments necessary to verify the suggestions offered, have proved so many and varied, that I venture in the meanwhile to describe the action of the poison on the salivary glands, leaving a discussion of the rationale of secretion to a future paper.

The necessity of using the inconvenient aqueous and alcoholic extracts of jaborandi has of late been obviated by the separation of its alkaloid pilocarpin. In these experiments I have used the nitrate of pilocarpin [1], a salt of the alkaloid very readily soluble in water.

The sub-maxillary gland of the dog was chosen for experi-

[1] I have, throughout, obtained it from Mr Martindale.

ment, owing to its exposed condition and to the comparative ease with which its nerves can be isolated; a few experiments were made on the parotid, but these were not increased in number, since there seems little reason to doubt that that which is true for one salivary gland is also with slight modifications true for the rest.

In every case the animal was placed under anæsthetics during the operation and killed at its close; the anæsthetics employed have been various, most frequently morphia or chloroform, occasionally chloral hydrate, or croton chloral hydrate.

In registering the amount of salivary secretion and of the blood-flow through the gland, a Ludwig's kymograph was used, against the slowly-moving paper of which lightly pressed three levers, in the same vertical line, each attached to the crosspiece of an electro-magnet, the lower one arranged in the usual way to mark seconds, the other two connected with a key and galvanic cell, so that when the key was put down a mark was made on the paper; one of the upper levers was used to register the amount of saliva, and the other the blood-flow, each key being put down for a definite quantity of fluid. In the case of the saliva a cannula tied in Wharton's duct was connected by a T piece with a tube divided into a number of equal parts; tubes of different calibre were used according to the nature of the experiment or at different stages of the experiment, so that the divisions, in all of about equal length, contained 1-8, 1-10, or 1-32 of a cubic centimetre. In observing the flow of blood, all the veins going to the jugular were tied, except the veins coming from the gland; then either the jugular was tied and cut across on the peripheral side of the ligature and the blood allowed to run into a narrow test-tube, of which each division was equal to ·5 cc; or a cut was made just at the division of the jugular, the jugular itself clamped, and the blood collected as before; in the latter case, when it was not necessary to record the blood-flow, a clamp was arranged so as just to pinch up the cut, and the clamp on the jugular taken off; in this way the blood returned to the heart and unnecessary loss was avoided.

By means of one of the keys the beginning and end of the

stimulation of a nerve, or of the injection of any substance, could also be recorded.

Occasionally the crural artery was connected with a manometer in the usual way, to be able to eliminate any effect from varying blood-pressure.

The pilocarpin was injected sometimes into the saphena vein and sometimes through the facial artery direct into the gland, in the manner described by Heidenhain (*Pflüger's Archiv*, Band v.), except that the subclavian arteries were not tied.

In every case the stimulus used was a Daniell's cell with a Du Bois Reymond's induction apparatus; with this the current was just perceptible to the tongue, when the secondary coil was at 12 or 13.

The effects of pilocarpin are, as will be seen, very different according to the amount of the dose.

A small quantity injected into the saphena vein causes in about thirty seconds a secretion of saliva and an increased flow of blood; both begin at about the same time, rapidly reaching a maximum, which is maintained for a varying short time; and both then decline slowly, and, as the secretion becomes less, still more slowly, so that it may be twenty, thirty, or more minutes before the normal condition of things is arrived at.

The minimum amount of pilocarpin which will produce secretion is very small, a few milligrams injected into the gland artery causing a marked flow of saliva.

The secretion is not affected by section of either the chorda tympani or the sympathetic nerve.

Stimulation of the peripheral end of the chorda tympani with currents of medium strength, gives an increase of secretion at whatever stage of the secretion it be stimulated. In one case, however, which certainly is not normal, a diminution of the flow was repeatedly caused by chorda tympani stimulation; the cause of this at present I can but guess at. Sometimes with rather strong currents the effect of chorda tympani stimulation was almost exactly that which would have been produced, had the poison not been acting, so that instead of adding its normal effects to those of the pilocarpin it merely increased those effects up to the normal stimulation amount. An experiment will shew this more clearly:

		Blood flow per minute $1^\circ = \frac{1}{8}$ c.c.	Saliva flow per minute $1^\circ = \frac{1}{8}$ c.c.
5·3	Normal	5°	—
5·5	Stimulated ch. ty. for 40″ sec. coil 11	rate 27°	12°
5·29	... ...	4°	—
5·30	Injected into facial artery towards the gland (not stopping the blood-supply) 6 minims of a 5 p.c. sol. pilocarpin.		
	The blood and saliva flow are influenced almost immediately, the former reaching its maximum rapidity in 20″, the latter in 30″	rate 22°	10°
5·40	... ...	10°	3°
5·42	Stimulated ch. ty. for 25″ sec. coil 11 ...	rate 27°	10°
5·45	... ...	8°	1°
5·47	Stimulated ch. ty. for 25″ sec. coil 11	rate 27°	8°

The above also shews the powerful effect that pilocarpin has on the gland; 17 mgrs. producing almost as great an effect as stimulation of the chorda tympani with the fairly strong current produced when the secondary coil is at 11.

Stimulation of the sympathetic during the increased flow by pilocarpin causes a slowing of the blood-flow and of the secretion, but I have not been able to stop the secretion by this means; the slowing probably depends on the lessened blood-supply.

The latent period of secretion with pilocarpin varies directly with the amount injected, just as it does with weak or strong chorda tympani stimulation.

If a second small quantity of pilocarpin be injected, the same results will follow as before, but unless some time has elapsed the effect is rather less in degree. The injection may be repeated several times with even feebler results, until a condition is reached in which there is a very slow continuous secre-

tion with a sub-normal blood-flow, and in which stimulation of the chorda tympani with whatever strength of current produces very slight effects on the saliva and comparatively slight on the blood-flow. But since before this stage has been reached, the pilocarpin has caused a very considerable fall of blood-pressure, and a weakening as well as a slowing of the heart, it is better to adopt another method of experiment, which will avoid as much as possible these sources of error, to see the effect of large doses. This may be done by injecting into the gland artery by Heidenhain's method, and operating in addition to observe the blood-flow, so that a great part of the pilocarpin may run out of the body[1].

Suppose that under such circumstances ·1 gm. of pilocarpin be injected, there will be for a short time, a minute or so, a rapid secretion, which rapidly will decline, so that in a few seconds later there will be a very slow secretion, perhaps of 1-32 cc. in two to three minutes, with a scanty blood-flow; stimulation of the chorda tympani will increase the secretion to perhaps 1-32 cc. in one minute, and produce a marked but comparatively slight increase in the blood-flow: subsequently the secretion becomes slower and slower, so that perhaps there is but 1-32 cc. saliva in five to ten minutes; which is unaltered by stimulation of the chorda tympani: finally the secretion stops altogether, and there is barely any blood-flow through the gland. Stimulation of the sympathetic still produces a secretion.

If now the animal be left a varying time the secretion starts again very slowly, but stimulation of the chorda tympani produces no effect; the flow slightly increases and the chorda tympani becomes faintly irritable, so that the saliva and the blood-flow are both increased, especially the latter, by stimulation of the nerve; these effects gradually become more marked until there is a fair secretion and fair nerve irritability.

We have seen that pilocarpin in not too large quantity produces just those effects which are produced by stimulating

[1] Even under these conditions it sometimes occurs that sufficient pilocarpin is carried to the heart to cause it to stop completely. If this appear imminent, the animal may be kept alive for some other experiment suggested by the circumstances by injecting 15 to 20 mgm. of atropin into a vein; the injection of atropin must not be deferred too long, or it will be useless.

the chorda tympani; we might therefore expect that after atropin has been given the effects of injecting a not too large quantity of pilocarpin would be similar to those of chorda tympani stimulation; this indeed is the case. It is well known that after atropin has been given stimulation of the chorda tympani causes an increase of blood-flow, but no longer a secretion of saliva; injection of pilocarpin into a vein at this stage gives an increase of blood-flow, but no secretion of saliva. This as a general statement is true, yet the matter is not quite so simple as might appear from such a statement, for when the chorda tympani is paralysed by atropin the increase of blood-flow from pilocarpin is very much less than that which would normally have been produced. This is, I believe, in part intimately connected with the fact that the increase of blood-flow by the injection of pilocarpin in such circumstances is generally less than that from chorda tympani stimulation, and in part is dependent upon the action of atropin. The effect of atropin on the vaso-dilator fibres of the chorda tympani has, I think, been rather underrated; in several experiments the following has been the course of events: on injecting a small quantity of atropin sulphate, say 5 mgr., the secretory power of the chorda tympani is lowered in the most marked manner, with little if any change in the vaso-dilator effects; on a further injection of say 2 mgr. the saliva rises slightly in fairly strong stimulation (sec. coil 10), the blood flowing rapidly but less than before. Although such a very striking diminution of the secretory effect of the chorda tympani is produced by the injection of 7 mgr. of atropin, yet to get rid of this small remaining effect atropin has to be injected to 10-16 mgr. The last doses of atropin produce an effect on the vaso-dilator fibres, so that when no secretion follows stimulation of the chorda tympani for 1′ 30″ with coil 8, the blood-flow, though strikingly increased, is much less than that normally produced.

It is this, I think, which largely causes the marked diminution in increase of blood-flow by pilocarpin after atropin.

It has been shown by Vulpian and others that atropin stops the salivary secretion which has been started by pilocarpin; this is easily verified, moreover the quantity required is relatively small, but the quantity required to paralyse the chorda

tympani when pilocarpin in small doses has been given is larger than that normally required, and so up to a certain point the greater the amount of pilocarpin given, the greater the amount of atropin necessary to paralyse the chorda tympani.

Vulpian has also stated[1] that after atropin has paralysed the chorda tympani, pilocarpin can cause no salivary secretion. With the heart of the frog[2] I have shown that atropin and jaborandi exert an action depending on their relative proportions, and that a heart freed from jaborandi stand-still by atropin can again be brought to a stand-still by jaborandi, and once more freed from it by atropin. A corresponding state of things holds good with the salivary gland: the pilocarpin secretion that has been stopped by atropin can be renewed by pilocarpin and again stopped by atropin; this may be proved by injecting into a vein, but is much more satisfactorily shown by injecting into the gland artery by the facial and allowing the first blood to flow out by the jugular. The following experiments will illustrate this antagonistic action:

		Saliva flow.
11.40.	Stimulated ch. ty. for 12 seconds. Sec. coil 11.	3° in 12″, *i.e.* rate 15° in 60″.
11.50.	Injected into saphena vein 17 mgm. atropin.	
12.0.	Stimulated ch. ty. Sec. coil 10 for 1 minute.	No trace.
12.25.	Stimulated ch. ty. Sec. coil 8 for 1 minute.	No trace.
12.30.	Injected into facial artery towards gland ·1 grm. pilocarpin. In 10 to 20 seconds the saliva begins to flow.	5° in 60″.
1.0.	Injected into saphena vein 20 mgrm. atropin.	stopped almost immediately.

1.15. Stimulated ch. ty. Good secretion.
1.17. Injected 43 mgr. pilocarpin into gland by artery. Almost immediately a rapid secretion, which rapidly becomes slow.
1.20. Injected into saphena vein 20 mgr. atropin. Secretion stopped.
1.25. Stimulated ch. ty. Sec. coil 9 for 1 minute. No trace of secretion.
1.35. Both ch. ty. still paralysed.

[1] Quoted by Nicolini, *Hist. des Pilocarpus*, 1876.
[2] *Journal of Anat. and Phys.* Oct. 1875.

1.40. Injected into gland by artery ·1 grm. pilocarpin. In about one minute secretion begins.
1.45. Saliva flow 2° in 40″.
1.46. Stimulated ch. ty. for 28″. Sec. coil 10. 2° in 28″.
2.10. Secretion stopped; both ch. ty. paralyzed, *i.e.* the pilocarpin has been washed out of the body, escaping by the jugular, so that the atropin remaining in the blood for the second time caused paralysis.

Summing up then the action of pilocarpin:

In *small doses, i.e.* up to about 30 mgr. It exerts an action on the gland very similar to that produced by stimulation of the chorda tympani.

It causes a rapid secretion, and a considerable increase of blood-flow; both secretion and blood-flow gradually declining.

Its effects are little if at all altered by section of the chorda tympani or of the sympathetic nerve.

Stimulation of the chorda tympani increases the pilocarpin effects, *i.e.* the nerve is functionally unaltered.

Stimulation of the sympathetic diminishes its effects, so that this nerve too is functionally unaltered.

The secretion is stopped by injecting atropin (a fact for some time known), but a quantity of atropin sufficient to paralyse the chorda tympani does *not* prevent a relatively large quantity of pilocarpin from producing its ordinary results. In fact, the secretion or absence of secretion is dependent on the relative quantity of the two poisons present, just as is the stand-still or beat of the heart.

In *larger doses*. Instead of causing a stronger saliva-flow, it causes none at all[1], and further prevents the chorda tympani from producing any secretion.

It considerably diminishes the blood-flow through the gland, as well as the effect of the chorda tympani on the blood-flow.

It does not however stop the sympathetic secretion. The action indeed is not very dissimilar to that of atropin; this agrees with its action on the vagus and inhibitory apparatus of the heart (*loc. cit.*), where in large doses it prevents any inhibition of the heart from stimulation of the vagus or of the junction of the sinus venosus, just as atropin does.

[1] The transient secretion ensuing immediately after injection is not here regarded as a proper effect of a strong dose, since the larger the dose the slighter and more transient it becomes.

From the *Journ. of Anat. and Phys.*, Vol. XI.]

ON THE DIGESTIVE FERMENT OF NEPENTHES.

By Sydney H. Vines, B.A., B. Sc., *Fellow of Christ's College, Cambridge.*

The publication of Dr Hooker's Address at the Belfast Meeting of the British Association for the Advancement of Science (August, 1874), and of Mr Darwin's book on *Insectivorous Plants*, has given rise to several investigations into the nature of the phenomena described in those works.

In the *Botanische Zeitung* for Oct. 29, 1875, Reess and Will of Erlangen published a series of experiments upon Drosera rotundifolia. They made a glycerin extract from the leaves, just in the same way as a glycerin extract is made from the glands of animals, and found that this extract, when acidified with very dilute hydrochloric acid, exercised a distinct digestive influence, causing complete solution of shreds of swollen-up fibrin within the space of eighteen hours. They found also that the filtrate from the fluid in which the fibrin had been dissolved gave the characteristic reaction of peptone when treated with caustic potash and copper sulphate (Biuret-reaction). They further found that the glycerine extract had naturally a feebly acid reaction, but that still no digestion of fibrin occurred when dilute hydrochloric acid was not added to the extract. By these experiments they clearly demonstrated the similarity, amounting to identity, of the phenomena which occur on the surface of the leaf of Drosera to those which take place in the digestive cavity of an animal. In both it appears that a ferment is secreted by the gland-cells, which is capable, in the presence of dilute acid, of converting proteids into peptones.

Similar experiments have been made by von Gorup-Besanez[1] with reference to Nepenthes. In this case, however, the secretion itself was the subject of experiment. Shreds of fibrin, prepared according to the method of Grünhagen, were rapidly attacked when exposed to the action of the secretion at a temperature of about 40° C., and the digestion was more

[1] *Berichte der deutsch-chem. Gesellschaft zu* Berlin. Jahrg. 9, No. 9. May 22, 1876.

rapid when dilute hydrochloric acid (0·2%) had been added. The filtered fluid gave the characteristic reaction of peptones, when treated with caustic potash and copper sulphate.

Contemporaneously with von Gorup-Besanez I had entered upon an investigation of the nature of the phenomena described by Dr Hooker as occurring in the pitchers of Nepenthes.

In my experiments upon Nepenthes (hybridus and gracilis) I followed the method pursued by Reess and Will in their experiments on Drosera, that is to say I made a glycerin extract of the pitchers. After having placed a shred of swollen-up fibrin in a small quantity of the extract, to which a few drops of dilute hydrochloric acid had been added, I found that, after eight hours at a temperature of 40° C. the filtrate gave a distinct peptone reaction, although the fibrin was not completely dissolved. I had also placed a similar shred of fibrin in a test-tube containing a small quantity of the dilute acid, and another in a test-tube containing a small quantity of the glycerin extract, which, I may add, was neutral in reaction. The filtrates from the fluids contained in these two tubes gave no trace of peptone when tested with caustic potash and copper sulphate.

These experiments show that in the gland-cells of the pitchers of Nepenthes, as in those of the leaves of Drosera, there is contained a digestive ferment which resembles that existing in the peptic glands of animals, in that it is soluble in glycerin, and in that it is capable of converting proteid into peptones in the presence of a sufficient quantity of acid.

In comparing the results of my experiments on the digestive power of a glycerine extract of Nepenthes pitchers, with those obtained by von Gorup-Besanez in his experiments with the secretion itself, I was struck by the great rapidity of the digestive process in the latter case, and I inferred that the quantity of ferment present in the glycerine extract must be very much smaller than that present in the secretion. Reference to similar experiments made upon the stomachs of animals shewed that Ebstein and Grützner[1] had found that a glycerin extract of much greater digestive power could be obtained from a gastric mucous membrane which had been previously treated with

[1] *Pflüger's Archiv*, Bd. VIII. p. 122—151. 1873.

dilute hydrochloric acid, than from a perfectly fresh one. The researches of Heidenhain[1] on the digestive ferment of the pancreas shew that from this organ also a more active glycerin extract could be obtained when it had been previously treated with a dilute acid. From his own experiments on the pancreas, and from those of Ebstein and Grützner on the stomach, he infers that these digestive ferments are not at first formed as such within the gland-cells. He regards the gland-cells as secreting an inert substance, which he terms zymogen, which may perhaps be a combination of the ferment with an albuminoid. It is only when this zymogen is decomposed, as a result of post-mortem change, or by the action of acids, that the ferment is liberated.

These investigations suggested that possibly the digestive ferment of Nepenthes might also be set free as a consequence of the decomposition of an inert body analogous to zymogen. Accordingly I treated some pitchers of Nepenthes hybridus and gracilis with dilute acetic acid (1%) for twenty-four hours previously to the preparation of the glycerin extract. On comparing the glycerin extract made from the pitchers so treated, with that made from fresh pitchers (gathered at the same time from the same plants), I found that the digestive power of the former greatly exceeded that of the latter. For instance, I placed a small pellet of swollen-up fibrin in a tube containing a small quantity of the acid extract, and a similar pellet in a tube containing a small quantity of the extract from the fresh pitchers. To each tube I added two cubic centimeters of dilute HCl. (·2%) and exposed them both to a temperature of 40°C. At the end of six hours the fibrin in the former tube had undergone complete solution, whereas that in the latter had decreased only slightly in size. The filtrates of both gave peptone reaction, though much more strongly in the first case than in the second.

Briefly summarising the results to which my experiments on Nepenthes lead, I find that, in the first place, they confirm those of von Gorup-Besanez, and those of Reess and Will, in the demonstration of the fact that "carnivorous" plants are capable of digesting proteid matters by a process which is essentially

[1] *Pflüger's Archiv*, Bd. x. p. 581. 1875.

similar to that by which the gastric digestion of animals is performed; and that, in the second place, they point out that the mode of origin of the digestive ferment, in Nepenthes at least, is essentially similar to that indicated by Heidenhain with reference to the digestive ferment of the pancreas (pancreatin), and by Ebstein and Grützner as regards that of the stomach (pepsin).

The foregoing is an abstract of a paper read before the Linnæan Society of London on June 15, 1876. Since that time I have more than once repeated my experiments, always with the same results. I have also followed the same line of investigation with reference to Sarracenia (flava), but I have failed, as yet, to obtain any indication of the presence of a digestive ferment in the pitchers of that plant.

I have also endeavoured to find out whether any diastatic ferment is present in the glands of these plants. In the case of Nepenthes the glycerin extract had no action upon starch, a result which von Gorup-Besanez also obtained in his experiments with the secretion.

In the case of Sarracenia I was surprised to find that a mixture of the glycerin extract with starch gave a well-marked sugar reaction. This I found to be due to the presence of sugar in the extract.

The fact that sugar occurred in the extract of Sarracenia, from which the digestive ferment was absent, as well as the fact that no sugar was found in the Nepenthes extract, in which the presence of the ferment was detected, seems to indicate that the pitchers of Sarracenia were in a condition in which their digestive function was in abeyance. Further experiments with this plant, we may hope, will shew that under other conditions the gland-cells of the plant, like those of Nepenthes, give rise to a digestive ferment.

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ON THE DEVELOPMENT OF THE SPINAL NERVES IN ELASMOBRANCH FISHES. By F. M. BALFOUR, B.A., *Fellow of Trinity College, Cambridge.* Plates I, II, III.

IN the course of an inquiry into the development of Elasmobranch Fishes, my attention has been specially directed to the first appearance and early stages of the spinal nerves, and I have been led to results which differ so materially from those of former investigators that I venture at once to lay them before the Society. I have employed in my investigations embryos of *Scyllium canicula, Scyllium stellare, Pristiurus,* and *Torpedo.* The embryos of the latter animal, especially those hardened in osmic acid, have proved by far the most favourable for my purpose, though, as will be seen from the sequel, I have been able to confirm the majority of my conclusions on embryos of all the above-mentioned genera.

A great part of my work was done at the Zoological Station founded by Dr Dohrn at Naples; and I have to thank both Dr Dohrn and Dr Eisig for the uniformly obliging manner in which they have met my requirements for investigation. I have more recently been able to fill up a number of lacunæ in my observations by the study of embryos bred in the Brighton Aquarium; for these I am indebted to the liberality of Mr Lee and the Directors of that institution.

The first appearance of the Spinal Nerves in Pristiurus.

In a *Pristiurus*-embryo, at the time when two visceral clefts become visible from the exterior (though there are as yet no openings from without into the throat), a transverse section through the dorsal region exhibits the following features (Plate I. fig. A):—

The external epiblast is formed of a single row of flattened elongated cells. Vertically above the neural canal the cells of

this layer are more columnar and form the rudiment of the primitively continuous dorsal fin.

The neural canal (*nc*) is elliptical in section, and its walls are composed of oval cells two or three deep. The wall at the two sides is slightly thicker than at the ventral and dorsal ends, and the cells at the two ends are also smaller than elsewhere. A typical cell from the side walls of the canal is about $\frac{1}{1900}$ inch in its longest diameter. The outlines of the cells are for the most part distinctly marked in the specimens hardened in either chromic or picric acid, but more difficult to see in those prepared with osmic acid; their protoplasm is clear, and in the interior of each is an oval nucleus very large in proportion to the size of its cell. The long diameter of a typical nucleus is about $\frac{1}{3000}$ inch, or about two-thirds of that of the cell.

The nuclei are granular, and very often contain several especially large and deeply stained granules; in other cases only one such is present, which may then be called a nucleolus.

In sections there may be seen round the exterior of the neural tube a distinct hyaline membrane: this becomes stained of a brown colour with osmic acid, and purple or red with hæmatoxylin or carmine respectively. Whether it is to be looked upon as a distinct membrane differentiated from the outermost portion of the protoplasm of the cells, or as a layer of albumen coagulated by the reagents applied, I am unable to decide for certain. It makes its appearance at a very early period, long before that now being considered; and similar membranes are present around other organs as well as the neural tube. The membrane is at this stage perfectly continuous round the whole exterior of the neural tube *as well on the dorsal surface as on the ventral.*

The section figured, whose features I am describing, belongs to the middle of the dorsal region. Anteriorly to this point the spinal cord becomes more elliptical in section, and the spinal canal more lanceolate; posteriorly, on the other hand, the spinal canal and tube become more nearly circular in section. Immediately beneath the neural tube is situated the notochord (*ch*). It exhibits at this stage a central area rich in protoplasm, and a peripheral layer very poor in protoplasm; externally it is invested by a distinct cuticular membrane.

Beneath the notochord is a peculiar rod of cells, constricted from the top of the alimentary canal[1]. On each side and below this are the two aortæ, just commencing to be formed, and ventral to these is the alimentary canal.

On each side of the body two muscle-plates are situated; their upper ends reach about one-third of the way up the sides of the neural tube. The two layers which together constitute the muscle-plates are at this stage perfectly continuous with the somatic and splanchnic layers of the mesoblast, and the space between the two layers is continuous with the body-cavity. In addition to the muscle-plates and their ventral continuations, there are no other mesoblast-cells to be seen. The absence of all mesoblastic cells dorsal to the superior extremities of the muscles is deserving of special notice.

Very shortly after this period, and, as a rule, before a third visceral cleft has become visible, the first traces of the spinal nerves make their appearance.

First Stage.—The spinal nerves do not appear at the same time along the whole length of the spinal canal, but are formed first of all in the neck and subsequently at successive points posterior to this.

Their mode of formation will be most easily understood by referring to Plate I. figs. B I, B II, B III, which are representations of three sections taken from the same embryo. B I is from the region of the heart; B II belongs to a part of the body posterior to this, and B III to a still posterior region.

In most points the sections scarcely differ from Plate I. fig. A, which, indeed, might very well be a posterior section of the embryo to which these three sections belong.

The chief point, in addition to the formation of the spinal nerves, which shows the greater age of the embryo from which the sections were taken, is the complete formation of the aortæ.

The upper ends of the muscle-plates have grown no further round the neural canal than in fig. A, and no scattered mesoblastic connective-tissue cells are visible.

In fig. A the dorsal surface of the neural canal was as com-

[1] *Vide* Balfour, *Preliminary Account of the Development of Elasmobranch Fishes*, Quart. Journ. of Microsc. Science, Oct. 1874, p. 33.

pletely rounded off as the ventral surface; but in fig. B III this has ceased to be the case. The cells at the dorsal surface of the neural canal have become rounder and smaller and begun to proliferate, and the uniform outline of the neural canal has here become broken (fig. B III, *pr*). The peculiar membrane completely surrounding the canal in fig. A now terminates just below the point where the proliferation of cells is taking place.

The prominence of cells which springs in this way from the top of the neural canal is the commencing rudiment of a pair of spinal nerves. In fig. B II, a section anterior to fig. B III, this formation has advanced much further (fig. B II, *pr*). From the extreme top of the neural canal there have now grown out two club-shaped masses of cells, one on each side; they are perfectly continuous with the cells which form the extreme top of the neural canal, and necessarily also are in contact with each other dorsally. Each grows outwards in contact with the walls of the neural canal; but, except at the point where they take their origin, they are not continuous with its walls, and are perfectly well separated by a sharp line from them.

In fig. B I, though the club-shaped processes still retain their attachment to the summit of the neural canal, they have become much longer and more conspicuous.

Specimens hardened in both chromic acid (Plate I. fig. C) and picric acid give similar appearances as to the formation of these bodies.

In those hardened in osmic acid, though the mutual relations of the masses of cells are very clear, yet it is difficult to distinguish the outlines of the individual cells.

In the chromic-acid specimens (fig. C) the cells of these rudiments appear rounded, and each of them contains a large nucleus.

I have been unable to prepare longitudinal sections of this stage, either horizontal or vertical, to show satisfactorily the extreme summit of the spinal cord; but I would call attention to the fact that the cells forming the proximal portion of the outgrowth are seen in every transverse section at this stage, and therefore exist the whole way along, whereas the *distal* portion is seen only in every third or fourth section, according

to the thickness of the sections. It may be concluded from this that there appears a continuous outgrowth from the spinal canal, from which discontinuous processes grow out.

In specimens of a very much later period (Plate III. fig. L) the proximal portions of the outgrowth are unquestionably continuous with each other, though their actual junctions with the spinal cord are very limited in extent. The fact of this continuity at a later period is strongly in favour of the view that the posterior branches of the spinal nerves arise from the first as a continuous outgrowth of the spinal cord, from which a series of distal processes take their origin. I have, however, failed to demonstrate this point absolutely. The processes, which we may call the nerve-rudiments, are, as appears from the later stages, equal in number to the muscle-plates.

It may be pointed out, as must have been gathered from the description above, that the nerve-rudiments have at this stage but one point of attachment to the spinal cord, and that this one corresponds with the dorsal or posterior root of the adult nerve.

The rudiments are, in fact, those of the posterior root only.

The next or second stage in the formation of these structures to which I would call attention occurs at about the time when three to five visceral clefts are present. The disappearance from the notochord in the anterior extremity of the body of a special central area rich in protoplasm serves as an excellent guide to the commencement of this epoch.

Its investigation is beset with far greater difficulties than the previous one. This is owing partly to the fact that a number of connective-tissue cells, which are only with great difficulty to be distinguished from the cells which compose the spinal nerves, make their appearance around the latter, and partly to the fact that the attachment of the spinal nerves to the neural canal becomes much smaller, and therefore more difficult to study.

Fortunately, however, in *Torpedo* these peculiar features are not present to nearly the same extent as in *Pristiurus* and *Scyllium*.

The connective-tissue cells, though they appear earlier in *Torpedo* than in the two other genera, are much less densely

packed, and the large attachment of the nerves to the neural canal is retained for a longer period.

Under these circumstances I consider it better, before proceeding with this stage, to give a description of the occurrences in *Torpédo*, and after that to return to the history of the nerves in the genera *Pristiurus* and *Scyllium*.

The development of the Spinal Nerves in Torpedo.

The youngest *Torpedo*-embryo in which I have found traces of the spinal nerves belongs to the earliest part of what I called the second stage.

The segmental duct[1] is just appearing, but the cells of the notochord have not become completely vacuolated. The rudiments of the spinal nerves extend half of the way towards the ventral side of the spinal cord; they grow out in a most distinct manner from the dorsal surface of the spinal cord (Plate I. fig. D a, *pr*); but the nerve-rudiments of the two sides are no longer continuous with each other at the dorsal median line, as in the earlier *Pristiurus*-embryos. The cells forming the proximal portion of the rudiment have the same elongated form as the cells of the spinal cord, but the remaining cells are more circular.

From the summit of the muscle-plates (*mp*) an outgrowth of connective-tissue has made its appearance (*c*), which eventually fills up the space between the dorsal surface of the cord and the external epiblast. There is not the slightest difficulty in distinguishing the connective-tissue cells from the nerve-rudiment. I believe that in this embryo the origin of the nerves from the neural canal was a continuous one, though naturally the peripheral ends of the nerve-rudiments were separate from each other.

The most interesting feature of the stage is the commencing formation of the anterior roots. Each of these arises (Plate I. fig. D a, *ar*) as a small but distinct outgrowth from the epiblast of the spinal cord, near the ventral corner of which it appears as a conical projection. Even from the very first it has an

[1] *Vide* Balfour, *Origin and History of Urino-genital Organs of Vertebrates*, Journal of Anatomy and Physiology, Oct. 1875.

indistinct form of termination and a fibrous appearance, while the protoplasm of which it is composed becomes very attenuated towards its termination.

The points of origin of the anterior roots from the spinal cords are separated from each other by considerable intervals. In this fact, and also in the nerves of the two sides never being united with each other in the ventral median line, the anterior roots exhibit a marked contrast to the posterior.

There exists, then, in *Torpedo*-embryos by the end of this stage distinct rudiments of both the anterior and posterior roots of the spinal nerves. These rudiments are at first quite independent of and disconnected with each other, and both take their rise as outgrowths of the epiblast of the neural canal.

The next *Torpedo*-embryo (Plate I. fig. D b), though taken from the same female, is somewhat older than the one last described. The cells of the notochord are considerably vacuolated; but the segmental duct is still without a lumen. The posterior nerve-rudiments are elongated, pear-shaped bodies of considerable size, and, growing in a ventral direction, have reached a point nearly opposite the base of the neural canal. They still remain attached to the top of the neural canal, though the connexion has in each case become a pedicle so narrow that it can only be observed with great difficulty.

It is fairly certain that by this stage each posterior nerve-rudiment has its own separate and independent junction with the spinal cord; their dorsal extremities are nevertheless probably connected with each other by a continuous commissure.

The cells composing the rudiments are still round, and have, in fact, undergone no important modifications since the last stage.

The important feature of the section figured (fig. D b), and one which it shares with the other sections of the same embryo, is the appearance of connective-tissue cells around the nerve-rudiment. These cells arise from two sources; one of these is supplied by the vertebral rudiments, which at the end of the last stage (Plate I. fig. C, *vr*) become split off from the inner layer of the muscle-plates. The vertebral rudiments have in fact commenced to grow up on each side of the neural

canal, in order to form the mass of cells out of which the neural arches are subsequently developed.

The dorsal extremities of the muscle-plates form the second source of these connective-tissue cells. These latter cells lie dorsal and external to the nerve-rudiments.

The presence of this connective-tissue, in addition to the nerve-rudiments, removes the possibility of erroneous interpretations in the previous stages of the *Pristiurus*-embryo.

It might be urged that the two masses which I have called nerve-rudiments are nothing else than mesoblastic connective-tissue commencing to develop around the neural canal, and that the appearance of attachment to the neural canal which they present is due to bad preparation or imperfect observation. The sections of both this and the last *Torpedo*-embryo which I have been describing clearly prove that this is not the case. We have, in fact, in the same sections the developing connective-tissue as well as the nerve-rudiments, and at a time when the latter still retains its primitive attachment to the neural canal. The anterior root (fig. D b, *ar*) is still a distinct conical prominence, but somewhat larger than in the previously described embryo; it is composed of several cells, and the cells of the spinal cord in its neighbourhood converge towards its point of origin.

In a *Torpedo*-embryo (Plate I. fig. D c) somewhat older than the one last described, though again derived from the oviduct of the same female, both the anterior and the posterior rudiments have made considerable steps in development.

In sections taken from the hinder part of the body I found that the posterior rudiments nearly agreed in size with those in fig. D b.

It is, however, still less easy than there to trace the junction of the posterior rudiments with the spinal cord, and the upper end of the rudiments of the two sides do not nearly meet.

In a considerable series of sections I failed to find any case in which I could be absolutely certain that a junction between the nerve and the spinal cord was effected; and it is possible that in course of the change of position which this junction undergoes there may be for a short period a break of continuity between the nerve and the cord. This, however, I do not think

probable. But if it takes place at all, it takes place before the nerve becomes functionally active, and so cannot be looked upon as possessing any physiological significance.

The rudiment of the posterior nerve in the hinder portion of the body is still approximately homogeneous, and no distinction of parts can be found in it.

In the same region of the body the anterior rudiment retains nearly the same condition as in the previous stage, though it has somewhat increased in size.

In the sections taken from the anterior part of the same embryo the posterior rudiment has both grown in size and also commenced to undergo histological changes by which it has become divided into a root, a ganglion and a nerve.

The root (fig. D c, *pr*) consists of small round cells which lie close to the spinal cord, and ends dorsally in a rounded extremity.

The ganglion (*g*) consists of larger and more elongated cells, and forms an oval mass enclosed on the outside by the downward continuation of the root, having its inner side nearly in contact with the spinal cord.

From its ventral end is continued the nerve, which is of considerable length, and has a course approximately parallel to that of the muscle-plate. It forms a continuation of the root rather than of the ganglion.

Further details in reference to the histology of the nerve-rudiment at this stage are given later in this paper, in the description of *Pristiurus*-embryos, of which I have a more complete series of sections than of the *Torpedo*-embryos.

When compared with the nerve-rudiment in the posterior part of the same embryo, the nerve-rudiment last described is, in the first place, considerably larger, and has, secondly, undergone changes, so that it is possible to recognize in it parts which can be histologically distinguished as nerve and ganglion.

The developmental changes which have taken place in the anterior root are not less important than those in the posterior. The anterior root now forms a very conspicuous cellular prominence growing out from the ventral corner of the spinal cord (fig. D c, *ar*). It has a straight course from the spinal cord to the muscle-plate, and there shows a tendency to turn downwards at

an open angle: this, however, is not represented in the specimen figured. The cells of which it is composed each contain a large oval nucleus, and are not unlike the cells which form the posterior rudiment. The anterior and posterior nerves are still quite unconnected with each other; and in those sections in which the anterior root is present the posterior root of the same side is either completely absent or only a small part is to be seen. The cells of the spinal cord exhibit a slight tendency to converge towards the origin of the anterior nerve-root.

In the spinal cord itself the epithelium of the central canal is commencing to become distinguished from the grey matter, but no trace of the white matter is visible.

I have succeeded in making longitudinal vertical sections of this stage, which prove that the ends of the posterior roots adjoining the junction with the cord are all connected with each other (Plate I. fig. D d).

If the figure representing a transverse section of the embryo (fig. D c) be examined, or better still the figure of a section of the slightly older *Scyllium*-embryo (Plate II. fig. H I or I I), the posterior root will be seen to end dorsally in a rounded extremity, and the junction with the spinal cord to be effected, not by the extremity of the nerve, but by a part of it at some little distance from this.

It is from these upper ends of the rudiments beyond the junction with the spinal cord that I believe the commissures to spring which connect together the posterior roots.

My sections showing this for the stage under consideration are not quite as satisfactory as is desirable; nevertheless they are sufficiently good to remove all doubt as to the presence of these commissures.

A figure of one of these sections is represented (Plate I. fig. D d). In this figure *pr* points to the posterior roots and *x* to the commissures uniting them.

In a stage somewhat subsequent to this I have succeeded in making longitudinal sections, which exhibit these junctions with a clearness which leaves nothing to be desired.

It is there effected (Plate III. fig. L) in each case by a protoplasmic commissure with imbedded nuclei[1]. Near its dorsal

[1] This commissure is not satisfactorily represented in the figure. *Vide* Explanation of Plate III.

extremity each posterior root dilates, and from the dilated portion is given off on each side the commissure uniting it with the adjoining roots.

Considering the clearness of this formation in this embryo, as well as in the embryo belonging to the stage under description, there cannot be much doubt that at the first formation of the posterior rudiments a continuous outgrowth arises from the spinal cord, and that only at a later period do the junctions of the roots with the cord become separated and distinct for each nerve.

I now return to the more complete series of *Pristiurus*-embryos, the development of whose spinal nerves I have been able to observe.

Second Stage of the Spinal Nerves in Pristiurus.

In the youngest of these (Plate II. fig. E) the notochord has undergone but very slight changes, but the segmental duct has made its appearance, and is as much developed as in the *Torpedo*-embryo from which fig. D b was taken.

(The embryo from which fig. E a was derived had three visceral clefts.)

There have not as yet appeared any connective-tissue cells dorsal to the top of the muscle-plates, so that the posterior nerve-rudiments are still quite free and distinct.

The cells composing them are smaller than the cells of the neural canal; they are round and nucleated; and, indeed, in their histological constitution the nerve-rudiments exhibit no important deviations from the previous stage, and they have hardly increased in size. In their mode of attachment to the neural tube an important change has, however, already commenced to be visible.

In the previous stage the two nerve-rudiments met above the summit of the spinal cord and were broadly attached to it there; now their points of attachment have glided a short distance down the sides of the spinal cord[1].

[1] [May 18, 1876. Observations I have recently made upon the development of the cranial nerves incline me to adopt an explanation of the change which takes place in point of attachment of the spinal nerves to the cord differing

The two nerve-rudiments have therefore ceased to meet above the summit of the canal; and in addition to this they appear in section to narrow very much before becoming united with its walls, so that their junctions with these appear in a transverse section to be effected by at most one or two cells, and are, comparatively speaking, very difficult to observe.

In an embryo but slightly older than that represented in fig. E a the first rudiment of the anterior root becomes visible. This appears, precisely as in *Torpedo,* in the form of a small projection from the ventral corner of the spinal cord (fig. E b, *ar*).

The second step in this stage (Plate II. fig. F) is comparable, as far as the connective-tissue is concerned, with the section of *Torpedo* (Plate I. fig. D d). The notochord (the histological details of whose structure are not inserted in this figure) is rather more developed, and the segmental duct, as was the case with the corresponding *Torpedo*-embryo, has become hollow at its anterior extremity.

The embryo from which the section was taken possessed five visceral clefts, but no trace of external gills.

In the section represented, though from a posterior part of the body, the dorsal nerve-rudiments have become considerably larger than in the last embryo; they now extend beyond the base of the neural canal. They are surrounded to a great extent by mesoblastic tissue, which, as in the case of the *Torpedo,* takes its origin from two sources, (1) from the commencing vertebral bodies, (2) from the summits of the muscle-plates.

It is in many cases very difficult, especially with chromic-acid specimens, to determine with certainty the limits of the rudiments of the posterior root.

In the best specimens a distinct bordering line can be seen; and it is, as a rule, possible to state the characters by which the cells of the nerve-rudiments and vertebral bodies differ. The more important of these are the following:—(1) The cells of the nerve-rudiment are distinctly smaller than those of the vertebral rudiment; (2) the cells of the nerve-rudiment are

from that enunciated in the text. I look upon this change as being apparent rather than real, and as due to a growth of the roof of the neural canal in the median dorsal line, which tends to separate the roots of the two sides more and more, and cause them to assume a more ventral position.]

elongated, and have their long axis arranged parallel to the long axis of the nerve-rudiment, while the cells surroudning them are much more nearly circular.

The cells of the nerve-rudiment measure about $\frac{1}{1600} \times \frac{1}{4600}$ to $\frac{1}{1600} \times \frac{1}{3200}$ inch, those of the vertebral rudiment $\frac{1}{1600} \times \frac{1}{1900}$ inch. The greater difficulty experienced in distinguishing the nerve-rudiment from the connective-tissue in *Pristiurus* than in *Torpedo* arises from the fact that the connective-tissue is much looser and less condensed in the latter than in the former.

The connective-tissue cells which have grown out from the muscle-plates form a continuous arch over the dorsal surface of the neural tube (*vide* Plate II. fig. F); and in some specimens it is difficult to see whether the arch is formed by the rudiment of the posterior root or by connective-tissue. It is, however, quite easy with the best specimens to satisfy one's self that it is from the connective-tissue, and not the nerve-rudiment, that the dorsal investment of the neural canal is derived.

As in the previous case, the upper ends of each pair of posterior nerve-rudiments are quite separate from one another, and appear in sections to be united by a very narrow root to the walls of the neural canal at the position indicated in fig. F.[1]

The cells forming the nerve-rudiments have undergone slight modifications; they are for the most part more distinctly elongated than in the earlier stage, and appear slightly smaller in comparison with the cells of the neural canal.

They possess as yet no distinctive characters of nerve-cells. They stain more deeply with osmic acid than the cells around them, but with hæmatoxylin there is but a very slight difference in intensity between their colouring and that of the neighbouring connective-tissue cells.

The anterior roots have grown considerably in length, but their observation is involved in the same difficulties with chromic-acid specimens as that of the posterior rudiments.

There is a further difficulty in observing the anterior roots, which arises from the commencing formation of white matter in the cord. This is present in all the anterior sections of the

[1] The artist has not been very successful in rendering this figure.

embryo from which fig. F is taken. When the white matter is formed the cells constituting the junction of the anterior nerve-root with the spinal cord undergo the same changes as the cells which are being converted into the white matter of the cord, and become converted into nerve-fibres; these do not stain with hæmatoxylin, and thus an apparent space is left between the nerve-root and the spinal cord. This space by careful examination may be seen to be filled up with fibres. In osmic-acid sections, although even in these the white matter is stained less deeply than the other tissues, it is a matter of comparative ease to observe the junction between the anterior nerve-root and the spinal cord.

I have been successful in preparing satisfactory longitudinal sections of embryos somewhat older than that shown in fig. F, and they bring to light several important points in reference to the development of the spinal nerves. Three of these sections are represented in Plate II. figs. G 1, G 2, & G 3.

The sections are approximately horizontal and longitudinal. G 1 is the most dorsal of the three; it is not quite horizontal though nearly longitudinal. The section passes exactly through the point of attachment of the posterior roots to the walls of the neural canal.

The posterior rudiments appear as slight prominences of rounded cells projecting from the wall of the neural canal. From transverse sections the attachment of the nerves to the wall of the neural canal is proved to be very narrow, and from these sections it appears to be of some length in the direction of the long axis of the embryo. A combination of the sections taken in the two directions leads to the conclusion that the nerves at this stage thin out like a wedge before joining the spinal cord.

The independent junctions of the posterior rudiments with the spinal cord at this stage are very clearly shown, though the rudiments are probably united with each other just dorsal to their junction with the spinal cord.

The nerves correspond in number with the muscle-plates, and each arises from the spinal cord, nearly opposite the middle line of the corresponding muscle-plates (figs. G 1 & G 2).

Each nerve-rudiment is surrounded by connective-tissue

cells, and is separated from its neighbours by a considerable interval.

At its origin each nerve-rudiment lies opposite the median portion of a muscle-plate (figs. G 1 & G 2); but, owing to the muscle-plate acquiring an oblique direction, at the level of the dorsal surface of the notochord it appears in horizontal sections more nearly opposite the interval between two muscle-plates (figs. G 2 & G 3).

In horizontal sections I find masses of cells which make their appearance on a level with the ventral surface of the spinal cord. I believe I have in some sections successfully traced these into the spinal cord, and I have little doubt that they are the anterior roots of the spinal nerves; they are opposite the median line of the muscle-plates, and do not appear to join the posterior roots (*vide* fig. G 3, *ar*).

At the end of this period or second stage the main characters of the spinal nerves in *Pristiurus* are the following:—

(1) The posterior nerve-rudiments form somewhat wedge-shaped masses of tissue attached dorsally to the spinal cord.

(2) The cells of which they are composed are typical undifferentiated embryonic cells, which can hardly be distinguished from the connective-tissue cells around them.

(3) The nerves of each pair no longer meet above the summit of the spinal canal, but are independently attached to its sides.

(4) Their dorsal extremities are probably united by commissures.

(5) The anterior roots have appeared; they form small conical projections from the ventral corner of the spinal cord, but have no connexion with the posterior rudiments.

The Third Stage of the Spinal Nerves in Pristiurus.

With the *third stage* the first distinct histological differentiations of the nerve-rudiments commence. Owing to the changes both in the nerves themselves and in the connective-tissue around them, which becomes less compact and its cells stellate, the difficulty of distinguishing the nerves from the

surrounding cells vanishes; and the difficulties of investigation in the latter stages are confined to the modes of attachment of the nerves to the neural canal, and the histological changes which take place in the rudiments themselves.

The stage may be considered to commence at the period when the external gills first make their appearance as small buds from the walls of the visceral clefts. Already, in the earliest rudiments of the posterior root of this period now figured, a number of distinct parts are visible (Plate II. fig. H 1).

Surrounding nearly the whole structure there is present a delicate investment similar to that which I mentioned as surrounding the neural canal and other organs; it is quite structureless, but becomes coloured with all staining reagents. I must again leave open the question whether it is to be looked upon as a layer of coagulated protoplasm or as a more definite structure. This investment completely surrounds the proximal portion of the posterior root, but vanishes near its distal extremity.

The nerve-rudiment itself may be divided into three distinct portions:—(1) the proximal portion, in which is situated the pedicle of attachment to the wall of the neural canal; (2) an enlarged portion, which may conveniently, from its future fate, be called the ganglion; (3) a distal portion beyond this. The proximal portion presents a fairly uniform diameter, and ends dorsally in a rounded expansion; it is attached remarkably enough, not by its extremity, but by its side, to the spinal cord. The dorsal extremities of the posterior nerves are therefore free; as was before mentioned, they probably serve as the starting-point of the longitudinal commissures between the posterior roots.

The spinal cord at this stage is still made up of fairly uniform cells, which do not differ in any important particulars from the cells which composed it during the last stage. The outer portion of the most peripheral layer of cells has already begun to be converted into the white matter.

The delicate investment spoken of before still surrounds the whole spinal cord, except at the points of junction of the cord with the nerve-rudiments. Externally to this investment, and separated from it for the most part by a considerable interval,

a mesoblastic sheath (Plate II. fig. H I, *i*) for the spinal cord is beginning to be formed.

The attachment of the nerve-rudiments to the spinal cord, on account of its smallness, is still very difficult to observe. In many specimens where the nerve is visible a small prominence may be seen rising up from the spinal cord at a point corresponding to *x* (Plate II. fig. H I). It is, however, rare to see this prominence and the nerve continuous with each other: as a rule they are separated by a slight space, and frequently one of the cells of the mesoblastic investment of the spinal cord is interposed between the two. In some especially favourable specimens, similar to the one figured, there can be seen a distinct cellular prominence (fig. H I, *x*) from the spinal cord, which becomes continuous with a small prominence on the lateral border of the nerve-rudiment near its free extremity. The absence of a junction between the two in a majority of sections is only what might be expected, considering how minute the junction is.

Owing to the presence of the commissure connecting the posterior roots, some part of a nerve is present in every section.

The proximal extremity of the nerve-rudiment itself is composed of cells, which, by their smaller size and a more circular form, are easily distinguished from cells forming the ganglionic portion of the nerve.

The ganglionic portion of the nerve, by its externally swollen configuration, is at once recognizable in all the sections in which the nerve is complete. The delicate investment before mentioned is continuous around it. The cells forming it are larger and more elongated than the cells forming the upper portion of the nerve-rudiment: each of them possesses a large and distinct nucleus.

The remainder of the nerve-rudiment forms the commencement of the true nerve. It can in this stage be traced only for a very small distance, and gradually fades away, in such a manner that its absolute termination is very difficult to observe.

The connective-tissue cells which surround the nerve-rudiment are far looser than in the last stage, and are commencing to throw out processes and become branched.

The anterior root-nerve has grown very considerable since the last stage. It projects from the same region of the cord as before, but on approaching the muscle-plate takes a sudden bend downwards (fig. H II, *ar*).

I have failed to prove that the anterior and posterior roots are at this stage united.

Fourth Stage.

In an embryo but slightly more advanced than the one last described, important steps have been made in the development of the nerve-rudiment. The spinal cord itself now possesses a covering of white matter; this is thickest at the ventral portion of the cord, and extends to the region of the posterior root of the spinal nerve.

The junction of the posterior root with the spinal cord is easier to observe than in the last stage.

It is still effected by means of unaltered cells, though the cells which form the projection from the cord to the nerve are commencing to undergo changes similar to those of the cells which are being converted into white matter.

In the rudiment of the posterior root itself there are still three distinct parts, though their arrangement has undergone some alteration and their characters have become more marked (Plate II. fig. I I).

The root of the nerve (fig. I I, *pr*) consists, as before, of nearly circular cells, each containing a nucleus, very large in proportion to the size of the cell. Its cells have a diameter of about $\frac{1}{3000}$ of an inch, and it forms not only the junction between the ganglion and the spinal canal, but is also continued into a layer investing the outer side of the ganglion and continuous with the nerve beyond the ganglion.

The cells which compose the ganglion (fig. I I, *sp. g*) are easily distinguished from those of the root. Each cell is elongated with an oval nucleus, large in proportion to the cell; and its protoplasm appears to be continued into an angular, not to say fibrous process, sometimes at one and more rarely at both ends. The processes of the cells are at this stage very difficult

to observe: figs. I a, I b, I c represent three cells provided with them and placed in the positions they occupied in the ganglion.

The relatively very small amount of protoplasm in comparison to the nucleus is fairly represented in these figures, though not in the drawing of the ganglion as a whole. In the centre of each nucleus is a nucleolus.

Fig. I b, in which the process points towards the root of the nerve, I regard as a commencing nerve-fibre: its more elongated shape seems to imply this. In the next stage special bundles of nerve-fibres become very conspicuous in the ganglion. The long diameter of an average ganglion-cell is about $\frac{1}{1600}$ of an inch. The whole ganglion forms an oval mass, well separated both from the nerve-root and the nerve, and is not markedly continuous with either. On its outer side lies the downward process of the nerve-root before mentioned.

The nerve itself is still, as in the last case, composed of cells which are larger and more elongated than either the cells of the root or the ganglion.

The condition of the anterior root at this stage is hardly altered from what it was; it is composed of very small cells, which with hæmatoxylin stain more deeply than any other cells of the section. A figure of it is given in I II.

Horizontal longitudinal sections of this stage are both easy to make and very instructive. On plate III. fig. K I is represented a horizontal section through a plate near the dorsal surface of the spinal cord: each posterior root is seen in this section to lie nearly opposite the anterior extremity of a muscle-plate.

In a more ventral plane (fig. K II) this relation is altered, and the posterior roots lie opposite the hinder parts of the muscle-plates.

The nerves themselves are invested by the hyaline membrane spoken of above; and surrounding this again there is present a delicate mesoblastic investment of spindle-shaped cells.

Longitudinal sections also threw light upon the constitution of the anterior nerve-roots (*vide* fig. K II. *ar*). In the two segments on the left-hand side in this figure the anterior roots are cut through as they are proceeding, in a more or less horizontal course, from the spinal cord to the muscle-plates.

Where the section (which is not quite horizontal) passes through the plane of the notochord, as on the right-hand side, the anterior roots are cut transversely. Each root, in fact, changes its direction, and takes a downward course.

The anterior roots are situated nearly opposite the middle of the muscle-plates: their section is much smaller than that of the posterior roots, and with hæmatoxylin they stain more deeply than any of the other cells in the preparation.

I have satisfied myself that by this stage the anterior and posterior roots have united.

The period now arrived at forms a convenient break in the development of the spinal nerves; and I hope to treat the remainder of the subject, especially the changes in the ganglion, the development of the ganglion-cells, and of the nerve-fibres, in a subsequent paper.

I will only add that, not long after the stage last described, the posterior root unites with the anterior root at a considerable distance below the cord: this is shown in Plate III. fig. L. Still later the portion of the root between the ganglion and the spinal cord becomes converted into nerve-fibres, and the ganglion becomes still further removed from the cord, while at the same time it appears distinctly divided into two parts.

As regards the development of the cranial nerves, I have made a few observations, which, though confessedly incomplete, I would desire to mention here, because, imperfect as they are, they seem to shew that in Elasmobranch Fishes the cranial nerves resemble the spinal nerves in arising as outgrowths from the central nervous system.

I have given a figure of the development of a posterior root of a cranial nerve in fig. M I. The section is taken from the same embryo as figs. B I, B II, and B III.

It passes through the anterior portion of a thickening of the external epiblast, which eventually becomes involuted as the auditory vesicle.

The posterior root of a nerve (VII) is seen growing out from the summit of the hind brain in precisely the same manner that the posterior roots of the spinal nerves grow out from the spinal cord: it is the rudiment of the seventh or facial nerve. The section behind this (fig. M II), still in the region of

the ear, has no trace of a nerve, and thus serves to show the early discontinuity of the posterior nerve-rudiments which arise from the brain.

I have as yet failed to detect any cranial anterior roots like those of the spinal nerves[1]. The similarity in development between the cranial and spinal nerves is especially interesting, as forming an important addition to the evidence which at present exists that the cranial nerves are only to be looked on as spinal nerves, especially modified in connexion with the changes which the anterior extremity of the body has undergone in existing vertebrates.

My results may be summarized as follows:—

Along the extreme dorsal summit of the spinal cord there arises on each side a continuous outgrowth.

From each outgrowth processes corresponding in number to the muscle-plates grow downwards. These are the posterior nerve-rudiments.

The outgrowths, at first attached to the spinal cord throughout their whole length, soon cease to be so, and remain in connexion with it in certain spots only, which form the junction of the posterior roots with the spinal cords.

The original outgrowth on each side remains as a bridge, uniting together the dorsal extremities of all the posterior rudiments. The points of junction of the posterior roots with the spinal cord are at first situated at the extreme dorsal summit of the latter, but eventually travel down, and are finally placed on the sides of the cord.

After these events the posterior nerve-rudiments grow rapidly in size, and become differentiated into a root (by which they are attached to the spinal canal), a ganglion, and a nerve.

The anterior roots, like the posterior, are outgrowths from the spinal cord; but the outgrowths to form them are from the first discontinuous, and the points from which they originally spring remain as those by which they are permanently attached to the spinal cord, and do not, as in the case of the posterior roots, undergo a change of position. The anterior roots arise,

[1] [May 18, 1876. Subsequent observations have led me to the conclusion that no anterior nerve-roots are to be found in the brain.]

not vertically below, but opposite the intervals between the posterior roots.

The anterior roots are at first quite separate from the posterior roots; but soon after the differentiation of the posterior rudiment into a root, ganglion, and nerve a junction is effected between each posterior nerve and the corresponding anterior root. The junction is from the first at some little distance from the ganglion.

Investigators have hitherto described the spinal nerves as formed from part of the mesoblast of the protovertebræ. HIS alone, so far as I know, takes a different view.

HIS'S[1] observations lead him to the conclusion that the posterior roots are developed as ingrowths from the external epiblast into the space between the protovertebræ and the neural canal. These subsequently become constricted off, unite with the neural canal and form spinal nerves.

These statements, which have not been since confirmed, diverge nearly to the same extent from my own results as does the ordinary account of the development of these parts.

HENSEN (VIRCHOW'S 'Archiv,' vol. xxxi. 1864) also looks upon the spinal nerves as developed from the epiblast, but not as a direct result of his own observations[2].

Without attempting, for the present at least, to explain this divergence, I venture to think that the facts which I have just described have distinct bearings upon one or two important problems.

One point of general anatomy upon which they throw considerable light is the primitive origin of nerves.

So long as it was admitted that the spinal and cerebral nerves developed in the embryo independently of the central nervous system, their mode of origin always presented to my mind considerable difficulties.

It never appeared clear how it was possible for a state of

[1] Erste Anlage des Wirbelthier-Leibes.

[2] [May 18, 1876. Since the above was written Hensen has succeeded in showing that in mammals the rudiments of the posterior roots arise in a manner closely resembling that described in the present paper; and I have myself, within the last few days, made observations which incline me to believe that the same holds good for the chick. My observations are as yet very incomplete.]

things to have arisen in which the central nervous system, as well as the peripheral terminations of nerves, whether motor or sensory, were formed independently of each other, while between them a third structure was developed which, growing in both directions (towards the centre and towards the periphery), ultimately brought the two into connexion.

That such a condition could be a primitive one seemed scarcely possible.

Still more remarkable did it appear, on the supposition that the primitive mode of formation of these parts was represented in the developmental history of vertebrates, that we should find similar structural elements in the central and in the peripheral nervous systems.

The central nervous system arises from the epiblast, and yet contains precisely similar nerve-cells and nerve-fibres to the peripheral nervous system, which, if derived, as is usually stated, from the mesoblast, was necessarily supposed to have a completely different origin from the central nervous system.

Both of these difficulties are to a great extent removed by the facts of the development of these parts in Elasmobranchs.

If it be admitted that the spinal roots develop as outgrowths from the central nervous system in Elasmobranch Fishes, the question arises, how far it can be supposed to be possible that in other vertebrates the spinal roots and ganglia develop independently of the spinal cord, and only subsequently become united with it.

I have already insisted that this cannot be the primary condition; and though I am of opinion that the origin of the nerves in higher vertebrates ought to be worked over again, yet I do not think it impossible that, by a secondary adaptation, the nerve-roots might develop in the mesoblast[1].

The presence of transverse commissures connecting the central ends of all the posterior roots is very peculiar. The commissures may possibly be looked on as outlying portions of the cord, rather than as parts of the nerves.

I have not up to this time followed their history beyond a

[1] [May 18, 1876. Hensen's observations, as well as those recently made by myself on the chick, render it almost certain that the nerves in all Vertebrates spring from the spinal cord.]

somewhat early period in embryonic life, and am therefore unacquainted with their fate in the adult.

As far as I am aware, no trace of similar structures has been met with in other vertebrates.

The commissures have a very strong resemblance to those by which in Elasmobranch Fishes the glossopharyngeal nerve and the branches of the pneumogastric are united in an early embryonic stage[1].

I think it not impossible that the commissures in the two cases represent the same structures. If this is the case, it would seem that the junction of a number of nerves to form the pneumogastric is not a secondary state, but the remnant of a primary one, in which all the spinal nerves were united, as they embryonically are in Elasmobranchs.

One point brought out in my investigations appears to me to have bearings upon the origin of the central canal of the Vertebrate nervous system, and in consequence upon the origin of the Vertebrate group itself.

The point I allude to is the posterior nerve-rudiments making their first appearance at the *extreme dorsal summit* of the spinal cord.

The transverse section of the ventral nervous cord of an ordinary segmented worm consists of two symmetrical halves placed side by side.

If by a mechanical folding the two lateral halves of the nervous cord became bent towards each other, while into the groove formed between the two the external skin became pushed, we should have an approximation to the Vertebrate spinal cord. Such a folding might take place to give extra rigidity to the body in the absence of a vertebral column.

If this folding were then completed in such a way that the groove, lined by external skin and situated between the two lateral columns of the nervous system, became converted into a canal, above and below which the two columns of the nervous system united, we should have in the transformed nervous cord an organ strongly resembling the spinal cord of Vertebrates.

This resemblance would even extend beyond mere external

[1] Balfour, *A Preliminary Account of the Development of Elasmobranch Fishes*, Q. J. Micros. Sc. 1874, plate xv. fig. 14, *v.g.*

form. Let the ventral nervous cord of the common earthworm, *Lumbricus agricola*, be used for comparison[1], a transverse section of which is represented by LEYDIG[2] and CLAPARÈDE. In this we find that on the ventral surface (the Annelidan ventral surface) of the nervous cord the ganglion-cells (grey matter) (*k*) are situated, and on the dorsal side the nerve-fibres or white matter (*h*). If the folding that I have supposed were to take place, the grey and white matters would have very nearly the relative situations which they have in the Vertebrate spinal cord.

The grey matter would be situated in the interior and surround the epithelium of the central canal, and the white matter would nearly surround the grey and form the anterior white commissure. The nerves would then arise, not from the sides of the nervous cord as in existing Vertebrates, but from its extreme ventral summit.

One of the most striking features which I have brought to light with reference to the development of the posterior roots, is the fact of their growing out from the extreme dorsal summit of the neural canal—a position analogous to the ventral summit of the Annelidan nervous cord. Thus the posterior roots of the nerves in Elasmobranchs arise in the exact manner which might have been anticipated were the spinal cord due to such a folding as I have suggested. The argument from the nerves becomes the stronger, from the great peculiarity in the position of the outgrowth, a feature which would be most perplexing without some such explanation as I have proposed. The central epithelium of the neural canal according to this view represents the external skin; and its ciliation is to be explained as a remnant of the ciliation of the external skin now found amongst many of the lower Annelids.

I have, however, employed the comparison of the Vertebrate and Annelidan nervous cords, not so much to prove a genetic relation between the two as to show the *à priori* possibility of the formation of a *spinal canal* and the *à posteriori* evidence we have of the Vertebrate spinal canal having been formed in the way indicated.

[1] The nervous cords of other Annelids resemble that of *Lumbricus* in the relations of the ganglion-cells of the nerve-fibres.
[2] Tafeln zur vergleichenden Anatomie, Taf. iii. fig. 8.

I have not made use of what is really the strongest argument for my view, viz. that the embryonic mode of formation of the spinal canal, by a folding in of the external epiblast, is the very method by which I have supposed the spinal canal to have been formed in the ancestors of Vertebrates.

My object has been to suggest a meaning for the peculiar primitive position of the posterior roots, rather than to attempt to explain in full the origin of the spinal canal.

EXPLANATION OF THE PLATES[1].

PLATE I.

Fig. A. Section through the dorsal region of an embryo of *Scyllium stellare*, with the rudiments of two visceral clefts. The section illustrates the general features at a period anterior to the appearance of the posterior nerve-roots.

nc, neural canal; *mp*, muscle-plate; *ch*, notochord; *x*, subnotochordal rod; *ao*, rudiment of dorsal aorta; *so*, somatopleure; *sp*. splanchnopleure; *al*, alimentary tract. All the parts of the section except the spinal cord are drawn somewhat diagrammatically.

Figs. B I, B II, B III. Three sections of a *Pristiurus*-embryo. B I is through the heart, B II through the anterior part of the dorsal region, and B III through a point slightly behind this. Drawn with a camera. (Zeiss CC ocul. 2.)

In B III there is visible a slight proliferation of cells from the dorsal summit of the neural canal.

In B II this proliferation definitely constitutes two club-shaped masses of cells (*pr*), both attached to the dorsal summit of the neural canal. The masses are the rudiments of the posterior nerve-roots.

In B I the rudiments of the posterior roots are of considerable length.

pr, rudiment of posterior roots; *nc*, neural canal; *mp*, muscle-plate; *ch*, notochord; *x*, subnotochordal rod; *ao*, dorsal aorta; *so*, somatopleure; *sp*, splanchnopleure; *al*, alimentary canal; *ht*, heart.

Fig. C. Section from a *Pristiurus*-embryo, slightly older than B. Camera. (Zeiss CC ocul. 2.) The embryo from which this figure was taken was slightly distorted in the process of removal from the blastoderm.

vr, rudiment of vertebral body. Other reference letters as in previous figures.

Fig. D a. Section through the dorsal region of a *Torpedo*-embryo with three visceral clefts. (Zeiss CC ocul. 2.) The section shows the formation of the dorsal nerve-rudiments (*pr*) and of a ventral anterior nerve-rudiment (*ar*), which at this early stage is not distinctly cellular.

[1] The figures on these Plates give a fair general idea of the appearance presented by the developing spinal nerves; but the finer details of the original drawings have in several cases become lost in the process of copying.

The figures which are tinted represent sections of embryos hardened in osmic acid; those without colour sections of embryos hardened in chromic acid.

ar, rudiment of an anterior nerve-root; *y*, cells left behind on the separation of the external skin from the spinal cord; *c*, connective-tissue cells springing from the summit of the muscle-plates. Other reference letters as above.

Fig. D b. Section from dorsal region of a *Torpedo*-embryo somewhat older than D a. Camera. (Zeiss CC ocul. 2.) The posterior nerve-rudiment is considerably longer than in fig. D a, and its pedicle of attachment to the spinal cord is thinner. The anterior nerve-rudiment, of which only the edge is present in the section, is distinctly cellular.

m, mesoblast growing up from vertebral rudiment; *sd*, segmental duct.

Fig. D c. Section from a still older *Torpedo*-embryo. Camera. (Zeiss CC ocul. 2.) The connective-tissue cells are omitted. The rudiment of the ganglion (*g*) on the posterior root has appeared. The rudiment of the posterior nerve is much longer than before, and its junction with the spinal cord is difficult to detect. The anterior root is now an elongated cellular structure.

g, ganglion.

Fig. D d. Longitudinal and vertical section through a *Torpedo*-embryo of the same age as D c.

The section shows the commissures uniting the posterior roots.

Plate II.

Fig. E a. Section of a *Pristiurus*-embryo belonging to the second stage. Camera. (Zeiss CC ocul. 2.) The section shows the constriction of the pedicle which attaches the posterior nerve-rudiments to the spinal cord.

pr, rudiment of posterior nerve-root; *nc*, neural canal; *mp*, muscle-plate; *vr*, vertebral rudiment; *sd*, segmental duct; *ch*, notochord; *so*, somatopleure; *sp*, splanchnopleure; *ao*, aorta; *al*, alimentary canal.

Fig. E b. Section of a *Pristiurus*-embryo slightly older than E a. Camera. (Zeiss CC ocul. 2.) The section shows the formation of the anterior nerve-root (*ar*).

ar, rudiment of the anterior nerve-root.

Fig. F. Section of a *Pristiurus*-embryo with the rudiments of five visceral clefts. Camera. (Zeiss CC ocul. 2.)

The rudiment of the posterior root is seen surrounded by connective-tissue, from which it cannot easily be distinguished. The artist has not been very successful in rendering this figure.

Figs. G 1, G 2, G 3. The longitudinal and horizontal section of an embryo somewhat older than F. The embryo from which these sections were taken was hardened in osmic acid, but the sections have been represented without tinting. G 1 is most dorsal of the three sections. Camera. (Zeiss CC ocul. 1.)

nc, neural canal; *sp. c*, spinal cord; *pr*, rudiment of posterior root; *ar*, rudiment of anterior root; *mp*, muscle-plate; *c*, connective-tissue cells; *ch*, notochord.

Fig. H I. Section through the dorsal region of a *Pristiurus*-embryo in which the rudimentary external gills are present as very small knobs. Camera. (Zeiss CC ocul. 2.)

The section shows the commencing differentiation of the posterior nerve-rudiment into root (*pr*), ganglion (*sp.g*), and nerve (*n*), and also the attachment of the nerve-root to the spinal cord (*x*). The variations in the size

and shape of the cells in the different parts of the nerve-rudiment are completely lost in the figure.

pr, posterior nerve-root; *sp.g*, ganglion of posterior root; *n*, nerve of posterior root; *x*, attachment of posterior root to spinal cord; *w*, white matter of spinal cord; *i*, mesoblastic investment to the spinal cord.

Fig. H II. Section through the same embryo as H I. (Zeiss CC ocul. 1.)

The section contains an anterior root, which takes its origin at a point opposite the interval between two posterior roots.

The white matter has not been very satisfactorily represented by the artist.

Figs. I I, I II. Two sections of a *Pristiurus*-embryo somewhat older than H. Camera. (Zeiss CC ocul. 1.)

The connective-tissue cells are omitted.

Figs. I a, I b, I c. Three isolated cells from the ganglion of one of the posterior roots of the same embryo.

Plate III.

Figs. K I, K. II. Two horizontal longitudinal sections through an embryo in which the external gills have just appeared. K I is the most dorsal of the two sections. Camera. (Zeiss CC ocul. 1.)

The sections show the relative positions of the anterior and posterior roots at different levels.

pr, posterior nerve-rudiment; *ar*, anterior nerve-rudiment; *sp.c*, spinal cord; *n.c*, neural canal; *mp*, muscle-plate; *mp'*, first-formed muscles.

Fig. L. Longitudinal and vertical section through the trunk of a *Scyllium*-embryo after the external gills have attained their full development. Camera. (Zeiss CC ocul. 1.)

The embryo was hardened in a mixture of chromic acid and osmic acid.

The section shows the commissures which dorsally unite the posterior roots, and also the junction of the anterior and posterior roots. The commissures are unfortunately not represented in the figure with great accuracy; their outlines are in nature perfectly regular, and not as in the figure, notched at the junctions of the cells composing them. Their cells are apparently more or less completely fused, and certainly not nearly so clearly marked as in the figure. The commissures stain very deeply with the mixture of osmic and chromic acid, and form one of the most conspicuous features in successful longitudinal sections of embryos so hardened. In sections hardened with chromic acid only they cannot be seen with the same facility.

sp.c, spinal cord; *gr*, grey matter; *w*, white matter; *ar*, anterior root; *pr*, posterior root; *x*, commissure uniting the posterior roots.

Figs. M I, M II. Two sections through the head of the same embryo as fig. B. M I, the foremost of the two, passes through the anterior part of the thickening of epiblast, which becomes involuted as the auditory vesicle. It contains the rudiment of the seventh nerve, VII. Camera. (Zeiss CC ocul. 2.)

VII, rudiment of seventh nerve; *au*, thickening of external epiblast, which becomes involuted as the auditory vesicle; *n.c*, neural canal; *ch*, notochord; *pp*, body-cavity in the head; *so*, somatopleure; *sp*, splanchnopleure; *al*, throat, exhibiting an outgrowth to form the first visceral cleft.

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THE GENERATIVE ORGANS OF THE PARASITIC ISOPODA. *By* J. F. BULLAR, B.A., *Trinity College.* (Pl. IV.)

(From the Zoological Laboratory.)

IN the following pages are recorded some observations on the generative organs of some of the parasitic Isopoda. My investigations were carried on last spring in Dr Dohrn's Zoological Station, and my best thanks are due to him for his help and advice, as well as to Dr Eisig for his kind assistance during my stay at Naples.

The species investigated are the following:—

Cymothoa æstroides (Risso).
Nerocila maculata (M. Edw.).
Nerocila bivittata (Risso).
Anilocra physodes (Lin.).
Anilocra mediterranea (M. Edw.).

With the exception of the last they were kindly determined for me by Prof. Heller. They are all hermaphrodite. The generative organs are essentially alike in all. To avoid future confusion it may be well to state at once that the organs are paired, and those of the two sides quite distinct. The animals during the development of the generative products pass through three distinct stages, which may be distinguished by the following characters.

In the first stage[1] they have externally the appearance of males. There is a double penis (Pl. IV. fig. 4 P.), situated in the median line of the ventral wall of the last thoracic segment. The internal parts of the generative organs on each side consist (Pl. IV. fig. 1) of the ovary and the testes and their ducts. The ovary and testes form a continuous gland, of which the ovary is the posterior simple tubular portion, while the testes appear anteriorly as three cæcal diverticula at its outer border. The oviduct, a widish tube continuous with the wall of the ovary, arises from the outer border of the ovary behind the testes, and runs to the anterior edge of the sixth thoracic segment, where, at this stage, it ends

[1] C. æstroides and A. mediterranea are the only species which I have obtained in this stage.

blindly. The vas deferens is continued from the posterior end of the ovary; it is much narrower than the oviduct; after running straight backwards for a short distance it turns outwards and downwards, and opens externally at the extremity of the penis of its side.

Between the first and second stage a moult takes place, and the penis being part of the skin is thrown of, and in the second stage there is no penis. Neither the vas deferens nor oviduct have an external opening.

In the third stage the vas deferens still remains closed, but the oviduct has acquired a slit-like aperture at the anterior edge of the sixth thoracic segment, just at the base of the posterior flap of the brood-pouch, which is present at this stage.

I will now describe each stage more fully.

In the first stage the generative gland is always comparatively small, owing to the incompletely developed state of the ovary. This may be very small, containing only a few young ova, or it may contain more numerous ova, some of a considerable size. Except in the case of the very youngest ovaries it is easily seen that the formation of the fresh ova takes place only along the outer border of the organ (Pl. IV. fig. 2).

The testes (Pl. IV. fig. 1 T.) at this stage are fully developed; they often contain numerous spermatozoa.

The spermatozoa are arranged in bundles (Pl. IV. fig. 6) with their heads all pointing one way, and are so disposed that the anterior end of the bundle is wedge-shaped. Each spermatozoon (Pl. IV. fig. 6) consists of a very long thin filament tapering to a point at the posterior extremity. The anterior extremity is thicker, and here a peculiarly twisted leaf-like appendage is attached to it (Pl. IV. fig. 6). The spermatozoa are perfectly motionless. Their average length is 1.15 Mm., and that of the appendage .04 Mm.

The bundles of spermatozoa may be seen in the act of making their way from the testes down the outer side of the ovary to the vas deferens. They always pass downwards head foremost.

The vas deferens (Pl. IV. fig. 1 VD.) is usually filled with spermatozoa, and, except at a very early stage, presents a fusiform enlargement near its lower end, which is crowded with

spermatozoa. The outer surface of this enlargement is generally covered with branched pigment-cells.

In the second stage the skin has been changed, and, as stated above, the oviduct and vas deferens are both closed externally.

The ovary has increased considerably in size, and frequently many of the ova are completely developed. Young ova are also being formed along the outer margin of the ovary.

The wall of the ovary is an exceedingly fine membrane, lined internally with a single layer of large flattened epithelium-cells (Pl. IV. fig. 5), each of which contains generally four, but sometimes three or two conspicuous nuclei. Both the protoplasm and nuclei of the cells are very granular, the latter usually containing one or more larger granules. As the young ova increase in size they move away from the germinal part of the ovary, pushing out its wall where they come in contact with it to suit their shape, so that at this stage the wall of the ovary loses its primitive even outline. Owing to these changes each ovum, when examined with a high power, appears to be surrounded by a ring of cells, but by careful focussing, it can be easily seen that these are the lining cells of the wall of the ovary.

The oviduct is lined internally with a single layer of flattened epithelium-cells, continuous with those lining the wall of the ovary, but differing from them in being smaller and in containing only a single nucleus. The oviduct is provided with an external layer of longitudinal muscular fibres, which are continued on each side along the outer border of the ovary. There are apparently no circular fibres.

The testes have not increased in size. They contain, as before, spermatozoa.

The vas deferens is also filled with spermatozoa, the enlargement at its lower end being usually crowded with them.

At this stage the skin of the ventral surface can, with care, be separated like a blister from the body wall, and beneath it the flaps of the brood-pouch are seen arising as small oval buds from near the bases of the legs. At first they are quite soft and flat, but as they increase in size they become thrown into numerous small wrinkles, and at the same time it becomes

more and more easy to remove the outer skin. While the brood-flaps are being developed a very delicate chitinous skin is formed over the ventral surface beneath them. The brood-flaps reach their full development in size before the loose outer skin is thrown off.

The hardening of the brood-flaps by the formation of an outer chitinous layer is probably a quick process, for in individuals in which, as sometimes happens, half the outer skin is shed at a time the uncovered flaps are quite hard, while those remaining covered are still soft. Probably the hardening of the brood-flaps helps to burst the outer skin.

The young flaps are covered externally by a flattened epithelium. The chitinous layer is formed subsequently, and is quite structureless.

In the third stage, which is attained on the shedding of the outer skin, described in the last stage, the animal possesses a completely developed brood-pouch, formed by the overlapping flaps. The skin of the ventral surface is exceedingly delicate, and is now protected by the brood-pouch. The ovaries at the first part of the stage are very large, and fill nearly the whole of the body-cavity, causing the ventral wall to protrude considerably.

Very soon, however, the eggs are laid, and it is therefore a rare thing to find an individual of this stage with the eggs still in the ovary. When the eggs are laid the shape of the body is altered, the ventral wall being now pressed close up to the dorsal surface.

The testes (Pl. IV. fig. 3 T.) still remain; they have not increased in size, and look withered and dry, though they occasionally contain a few bundles of spermatozoa.

The vasa deferentia (Pl. IV. fig. 3 VD.), especially their enlargements, are still filled with spermatozoa.

The manner in which the ova are fertilised is a point which I have not as yet been able to determine satisfactorily. The oviduct only opens externally at the time when the brood-pouch is present, and as its opening is situated inside the brood-pouch it seems quite impossible that spermatozoa could be introduced into it by another animal.

There is often not more than one individual on a fish, and

as these solitary individuals may have embryos in their brood-pouches, they must either have fertilised their own ova, or be parthenogenetic, for they cannot be imagined to pass from one fish to another; indeed for one species at least, C. œstroides, which cannot swim, this is impossible.

Now self-impregnation, if it occurs, must be internal, for the vas deferens becomes closed before the eggs are laid, and remains so until after their development is complete.

Of course if self-impregnation occurs in these cases it occurs always. We have already seen that from the position of the external opening of the oviduct, the ova of one animal cannot be impregnated by another before they are laid. Therefore the only way in which we can imagine that a cross occurs is by supposing that self-fertilisation does not act until after the eggs have been laid, and that the spermatozoa of another individual are introduced by some means, at present unknown, into the brood-pouch, and have a prepotent effect.

It should be remembered that the brood-flaps overlap a great deal, and are not capable of being moved, and also that the spermatozoa are immoveable. These facts make it difficult to understand how the spermatozoa could by any possibility be introduced.

These animals show perhaps better than any others the manner in which hermaphroditism is acquired.

I think no one can doubt that all the Isopoda have descended from a common bisexual stock, and that the ancestors of the present parasitic species when they began to be parasitic were bisexual. It is evident that their hermaphroditism is the effect and not the cause of their habits. If a free form varied so as to be hermaphrodite, it would have, as far as we can see, no advantage over the bisexual forms, and would not therefore tend to be preserved. On the other hand, it is of such immense advantage to a parasitic animal to be hermaphrodite that such a variation would be almost certain to be preserved.

In the present case hermaphroditism was probably gained by the occurrence of a sport. The following considerations seem to show clearly that it was not the result of gradual modification.

The internal generative organs of the hermaphrodites re-

semble exactly the combined male and female organs of the free forms, such as Assellus aquaticus, described by G. O. Sars (*Crust. d'eau douce*). It is hardly credible that this would be the case if gradual modification either in the males or females had taken place.

The same argument applies to the external brood-pouch and penis, which are identical with those found in the free forms.

From the analogy of vertebrates it is reasonable to conclude that every embryo contains parts capable of developing into the generative organs of both sexes, and it is conceivable that from these parts both sets of organs may in certain cases become developed and functional. If we imagine such a sport to have been developed in one of the parasitic ancestors of the present animals, and to have produced some individuals like itself, it is all that is required to account for the hermaphroditism of the existing parasitic Isopoda.

DESCRIPTION OF THE FIGURES.

T. Testes. V.D. Vas deferens. S. Spermatozoa. O. Ovary. P. Penis.

Fig. I. Generative organs of A. mediterranea. First stage. The whole of the vas deferens and oviduct are not shewn.

Fig. II. Generative organs of C. æstroides. First stage. The vas deferens and one of the testss are not shewn.

Fig. III. Generative organs of A. physodes. Third stage—after the eggs have been laid.

Fig. IV. Penis and part of ventral wall of last thoraic segment of C. æstroides.

Fig. V. Cells from wall of ovary. First stage.

Fig. VI. Spermatozoa of A. mediterranea; only the anterior parts are represented.

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ON THE EARLY STAGES OF DEVELOPMENT OF THE NERVES IN BIRDS.

By A. MILNES MARSHALL, B.A., B.Sc., *St John's College*. (Plates v. and vi.)[1]

(From the Zoological Laboratory.)

IN the investigations here recorded, which are concerned almost exclusively with the early stages of development of the nerves, chick-embryos, incubated by the hen, and of ages varying from 36 hours to 4 days, were employed. These were hardened by immersion in picric acid—prepared after Kleinenberg's method—for 3 to 5 hours; and then transferred to alcohol of about 30 p. c., which was gradually increased in strength till absolute. As a staining agent Kleinenberg's preparation of hæmatoxylin was used.

Some specimens were hardened in chromic acid in the usual manner; but these have not proved nearly so satisfactory as picric acid specimens, and have only been used to confirm and control the results obtained from the latter: in fact, it is to the use of picric acid, as a hardening agent, that the results obtained are believed to be in great measure due. Very good results were obtained from duck-embryos, hardened in picric acid.

My observations, though I believe differing widely from any previous account of the chick, will be found to agree remarkably closely with Balfour's researches on the mode of development of the nerves in Elasmobranchs[2]. I mention this at once, as I shall have occasion to refer repeatedly to Balfour's paper.

Owing to the absence of protovertebræ, and to the mesoblast being less compact in the head, the early stages of development of the cranial nerves are more easily studied than those of the spinal, and will therefore be considered first.

Plate v. fig. 1, represents a transverse section through

[1] An abstract of this paper was read before the Royal Society on March 8th.
[2] Reprinted antea p. 54 from *Phil. Trans.* Vol. CLXVI. Pt. 1.

the hind-brain of a 43 hours chick. The exact position of the section can be defined, since it passes above through the deepest portion of the commencing auditory involution, *aud.*; while below it passes through the posterior part of the heart, only a short distance in front of the point of union of the omphalo-meseraic veins.

The external epiblast, *ep.*, is seen to be very thin over the summit of the neural canal, where it consists of a single layer of somewhat flattened cells. Towards the sides it thickens rapidly, and is pushed in slightly from the exterior so as to form on either side a shallow depression lined by a thick layer of epiblast, of which the cells are elongated vertically, and arranged in one layer at the margin of the depression, but towards the centre in two or three layers. This depression, *aud.*, is the commencing auditory involution, which at this period has the form of a wide shallow pit, through the deepest portion of which, as noticed above, the section passes.

This layer of epiblast is seen to lie in close contact with the walls of the neural canal for some distance on either side; while between the top of the neural canal and the epiblast there is a considerable interval.

The hind-brain at this period is of considerable length, and presents three or more dilatations separated by slight constrictions. The section figured passes through the second of these dilatations a little way behind its centre. In section the brain at this point is seen to be nearly circular in outline; and the central canal, which is of considerable size, is also approximately circular. The walls of the canal are thicker at the sides than at the top or bottom, and consist of elongated cells arranged radially, and placed three or four deep. At the extreme summit of the canal, however, the cells are seen to alter their shape, becoming slightly smaller, and nearly circular in outline. These spherical cells grow upwards, and spread out on either side, forming a mass, *m.*, which occupies the interval above alluded to, between the top of the canal and the external epiblast.

This mass consists throughout of cells identical with those at the extreme summit of the cord, and differing markedly by their smaller size and more spherical shape both from the cells

composing the rest of the brain-wall, and from the superficial epiblast-cells.

As is evident from the figure, there are no mesoblast-cells anywhere near from which the mass (m) could possibly be derived, owing to the superficial epiblast being in contact with the sides of the brain for a considerable distance on either side. Moreover, we shall see that the cells of the mass differ in appearance very much from mesoblast-cells; while, finally, an examination of sections taken in parts where the mass (m) is much smaller, demonstrates conclusively that the cells composing it do really arise in the manner described above, *i.e.* as outgrowths from the extreme summit of the neural canal. This outgrowth forms the earliest stage in the development of the cranial nerves; and the stage here represented may well be compared with Balfour's figures, see Pl. XI. B. 2 and 3.

Pl. V. fig. 2, represents a section from the same embryo, taken a little further forward than fig. 1. It passes through the anterior edge of the auditory depression, which is hardly recognizable in the section, except by the thickening of the epiblast at the sides of the neural canal.

The mass (m) is somewhat larger than in fig. 1, and has grown outwards so as to form on either side an oval mass which indents the upper wall of the hind-brain; this indentation is visible in fig. 1, but is a much more prominent feature in fig. 2; it has the effect of causing the general contour of the brain together with the outgrowing mass to appear tolerably uniform, so that in imperfectly preserved specimens the presence of the outgrowth might easily be overlooked.

The outgrowths (m) are still connected with the extreme summit of the brain, though the connection is somewhat narrower than in the preceding stage. The outgrowths of the two sides are manifestly continuous with one another across the top of the neural canal.

The cells composing the outgrowths have not altered in appearance. Owing partly to their increased lateral extension, and partly to the alteration in contour of the external epiblast, due to the slight development of the auditory depression, the outgrowths lie very close to the mesoblast. It is now seen that the mesoblast-cells are larger than the cells forming the out-

growths, from which they differ also in being more loosely arranged, very irregular in shape and size, and in almost invariably giving out one or more processes, often of considerable length.

The neural canal has altered its shape: instead of being circular it is now somewhat oval, with the long diameter vertical: this change is still more marked in the next figure.

Pl. v. fig. 3, is drawn from a section taken a very short distance in front of that represented in fig. 2, only two thin sections intervening. In it the outgrowths (*m*) have become much larger, and have grown downwards considerably on either side. Partly in consequence of this downgrowth, and partly owing to the external epiblast presenting only very slight lateral thickenings, the outgrowth on either side is in very extensive contact at its distal end with the mesoblast-cells: the two forms of cells are seen to differ widely from one another in the manner just noticed.

The outgrowths are still attached to the brain at its extreme summit only[1], and those of the two sides are still widely continuous with one another across the top of the neural canal.

Some of the cells of the outgrowth are seen to have altered their shape slightly, becoming oval instead of circular in outline.

Between figs. 1 and 2 four sections intervene, all showing the outgrowth (*m*), which is found to increase in size as we pass forwards from fig. 1 till we get to fig. 3, where it attains its maximum. In front of fig. 3 it rapidly gets smaller and almost completely disappears. At about the middle of the most anterior of the dilatations presented by the hind-brain, the outgrowth again becomes prominent, but assumes a slightly different form, shown in Pl. v. fig. 4. In this figure the superficial epiblast is seen to be very thin in its whole extent, but is still thinnest over the summit of the canal. The hind-brain (*hb*) is considerably larger than it was further back: the outgrowth of spherical cells from its summit occurs in the same manner as it did in fig. 3: the lateral processes (*m*) are, however, much more slender, and have grown much further down than in fig. 3. The mesoblast-cells present the same characters

[1] The morphological importance of this attachment is very clearly stated by Balfour, *op. cit.* p. 77.

as they did further back; but have grown round so as to lie between the outgrowth (*m*) and the superficial epiblast.

An examination of the sections immediately behind that represented in fig. 1, shows that the outgrowth (*m*) gets slightly smaller at first, then begins to dilate again, getting more and more prominent, till finally it attains the form shown in Pl. v. fig. 5. This section, which passes below through the anterior part of the mid-gut, is completely behind the auditory depression: the external epiblast is seen to be thin in its whole extent, but especially so over the summit of the neural canal, which is oval in section and smaller than in the preceding figures. The outgrowth (*m*) is very prominent, and extends outwards some distance on either side; its limits are very sharply defined, though peripherally it is in extensive contact with the surrounding mesoblast-cells. The section passes through the most anterior protovertebra; and it is with the part of the muscle-plate (*mp*) nearest the neural canal that the outgrowth (*m*) comes in contact. The cells of the muscle-plate are elongated and fusiform, and differ widely from those of the outgrowth; which latter has a tendency to grow out horizontally so as to lie between the muscle-plate and the superficial epiblast.

We thus see that towards the latter part of the second day the cells along the median dorsal line of the hind-brain become slightly smaller and more spherical than those making up the rest of the wall of the neural canal; and that these spherical cells grow upwards, so as to form a prominent outgrowth immediately beneath the external epiblast, and between it and the top of the brain. Since this outgrowth is visible in some form or other in all the sections taken through the hind-brain, it follows that it is a continuous growth, in the form of a longitudinal ridge extending the whole length of the hind-brain. This ridge is more prominent at the posterior part of the hind-brain than it is anteriorly, where it gradually decreases in size and disappears[1]. At intervals along its length the ridge

[1] I have not always been able to detect an actual outgrowth in *all* the sections between the points indicated by Plate v. figs. 3, 4; as the brain in this situation lies exceedingly close to the external epiblast. The cells along the

becomes more prominent, growing out into paired lateral processes. These processes are found, by a study of their later stages of development, to be the earliest rudiments of the cranial nerves.

Of those already described, the prominent outgrowth (*m*), seen in Pl. v. fig. 3, is the commencement of the facial and auditory nerves.

The outgrowth shown in fig. 4 is the fifth nerve; which, though longer than the preceding, is at this period much more slender.

Behind the ear we have a conspicuous outgrowth of considerable length, which gradually increases in prominence from before backwards, and attains its maximum in Pl. v. fig. 5. This subsequently gives origin to the glossopharyngeal and vagus nerves, and may be spoken of as the vagus-mass.

The sections posterior to that represented in fig. 5 show that the longitudinal ridge just described is not confined to the hind-brain, but extends backwards without any break for a certain distance down the spinal cord. As was the case in the brain, this ridge gives off at intervals paired processes, which grow outwards from the summit of the cord. These intervals correspond in number with the protovertebræ, and the processes themselves we shall find develope into the posterior roots and ganglia of the spinal nerves.

Pl. v. fig. 6, represents a transverse section through the posterior part of the most anterior protovertebra of the same embryo, from which figs. 1—5 were taken; the section passing through one of the posterior roots (*m*). The external epiblast (*ep*) is very thin: the spinal cord presents in section a characteristic oval shape. The cells at the top of the cord become, as was the case in the brain, somewhat smaller and more spherical, and grow outwards on either side into a long slender process. Though this process comes in contact with the mesoblast-cells of the protovertebræ, yet its outline is sharp and definite, and there is at this stage not the slightest difficulty in determining the limits of the outgrowth, or whether any given cell belongs to the nerve-root or to the mesoblast.

median dorsal line have however the characteristic spherical shape in all the sections.

The nerve-root (m) lies on either side close beneath the external epiblast: its distal end lies *outside* the muscle-plate, between it and the external epiblast. This relation, which is shown to a slighter extent, as regards the vagus, in Pl. v. fig. 5, is a characteristic feature of the upper (cervical) spinal nerves, and will be referred to again further on.

It is also seen that the nerve-root is larger on one side than on the other; and that the side on which it is larger is that on which the muscle-plate is less developed. The section, in fact, passes on the left side through the hinder part of the muscle-plate, but on the right side passes almost exactly through the interval between the first and second protovertebræ. Horizontal sections also show that the posterior roots do not lie opposite the centres of the protovertebræ, but are at first situated opposite their posterior halves; while in the case of a few of the upper (cervical) spinal nerves they extend further back, so as to overlap the anterior parts of the succeeding protovertebræ. The length of attachment to the cord of the posterior root of each spinal nerve is at first equal to about half a protovertebra.

The development of the posterior roots of the spinal nerves in the hinder part of the body resembles that just described as occurring in the anterior portion in its more important points, but presents some minor differences. The nerve rudiments are from the first much more slender than is the case further forwards. The spinal cord lies very close underneath the superficial epiblast, and, though the cells at the summit of the cord are always more spherical than the rest, I have not been able to satisfy myself of the presence of a continuous outgrowth, but am inclined to think that the nerves arise in pairs direct from the cord itself. The longitudinal extent of the attachments of the roots to the cord also seems to be less in the posterior spinal nerves.

Pl. v. fig. 7, represents a transverse section through the dorsal region of a 3-day chick, and illustrates the next stage in the development of the posterior spinal roots. The spinal cord has, relatively to the muscle-plates, grown considerably, both laterally and vertically: owing to the vertical increase—which gives rise to a broad ridge along the back of the embryo—the position of the posterior roots has somewhat altered; instead of

projecting out laterally as they did in fig. 6, they now lie against the sides of the spinal cord: owing to this change in position their tendency to run outside the muscle-plates, noticed in the earlier stages, no longer exists. The nerve-roots are also seen to be much smaller relatively to the cord than in their earlier stages, which would obviously facilitate the change in their position.

Though unequal growth of the surrounding parts may possibly be sufficient to account for the change of position of the spinal nerves, I cannot regard it as satisfactory so far as the vagus is concerned, for, in one series of specimens, sections taken in the same position as that represented in Pl. v. fig. 5, but at a rather later date, show that the tendency of the vagus to pass outside the muscle-plates is so decided that, in order to enable the nerve to subsequently pass inside the muscle-plate, some further explanation than a simple change in the relative rates of growth of different parts seems necessary: unless, indeed—and some of my specimens point very strongly to this conclusion—a part, at least, of the nerve remains permanently outside the muscle-plate, and just beneath the external epiblast.

The small size of the roots, (*m*) fig. 7, relatively to the spinal cord, is worth notice, as it shows that at this period the nerves grow relatively more slowly than the cord.

Another important feature is the point of attachment of the roots to the cord: this is no longer to the extreme summit, but to the angle between the top and sides of the cord; so that there is, at this stage, no evident outgrowth of cells from the summit of the cord. Later on we shall find the attachment shifting still further down the sides. The manner in which this change of attachment occurs is a matter of some uncertainty; but as far as the present stage is concerned, I am convinced that the explanation first proposed by Balfour[1] is correct, viz., that the shifting is apparent rather than real; and is due to rapid growth of the cells of the extreme top of the cord, which would have the effect of separating the roots, and, as it were, forcing them further apart.

[1] *Op. cit.* p. 64 and 65.

The last point to be noticed in fig. 7 is the appearance of a certain number of detached mesoblast-cells at the summit of the muscle-plate, and lying outside the nerve-root: these are shown on the right side only of the section: in appearance they do not differ much from the cells composing the nerve-root, from which however they may be distinguished by their more irregular shape, by their tendency to give out processes, and by their staining less deeply with hæmatoxylin.

The next stage is represented in Pl. v. fig. 8, drawn from a section through the posterior dorsal region of a 4-day chick embryo. It differs from fig. 7 in several points, the more important of which are the following:—The slope of the back, due mainly to vertical increase of the spinal cord, is rather steeper. The posterior roots (*m*) are considerably larger, and have grown down on each side in close contact with the spinal cord, between it and the muscle-plates. The point of attachment of the roots is difficult to determine accurately; their upper or proximal parts are very slender, and in many cases it is impossible to trace any connection between them and the cord, against which they lie. The distal part of the nerve swells out considerably, forming an oval enlargement—the spinal ganglion (*g*). The section also shows that the ganglia do not lie opposite the centres of the muscle-plates, but almost opposite the intervals between successive pairs.

Another important point is the comparative difficulty now met with in distinguishing between the cells of the nerve-root and the adjacent mesoblast-cells. Many of the cells of the nerve and ganglion are no longer spherical, but more or less elongated; while the mesoblast-cells are slightly smaller, and much more closely packed together than they were at first; while many of them no longer give out processes, but are spherical or fusiform in shape, and almost indistinguishable from some of the cells of the nerve-root. The mesoblast-cells have also grown all round the top of the spinal cord, forming a distinct layer between it and the external epiblast; while some of them have grown in between the sides of the spinal cord and the nerve-roots. Consequently, while the limits of the nerve-roots were perfectly easy to define in the early stages, even when there was extensive contact between them and the meso-

blast-cells; in the later stages the exact limits become very difficult, or even impossible, to fix, and certain cells near the periphery of the nerve-root, especially those near its distal end, might be epiblast-cells belonging to the root, or mesoblast-cells. It follows therefore that while it appeared certain that the growth of the nerve in its earlier stages was effected by multiplication of the cells of the original outgrowth, and consequently of epiblastic origin; in the later stages it is impossible to determine whether the nerve can still be described as a structure of purely epiblastic origin, or whether its growth is due in part to conversion of the adjacent mesoblast-cells.

With the structure of the spinal cord we are not directly concerned, but the presence of large numbers of spherical, or nearly spherical, cells in its substance is shown in the figure.

To recapitulate. The longitudinal ridge described in the hind-brain, as formed by an outgrowth of cells from the extreme summit of the neural canal, is continued down the spinal cord for a certain distance, but becomes inconspicuous in the hinder part of the body, where its presence has not been definitely ascertained. We find further that this ridge gives off paired processes opposite the posterior half of each protovertebra, these processes being the rudiments of the posterior roots of the spinal nerves: that these processes at first grow outwards just beneath the external epiblast; but subsequently, owing to changes in the shape of the embryo caused by unequal growth of different parts, alter their direction somewhat, and pass downwards between the muscle-plates and the spinal cord: that the proximal portion of each process becomes more slender, and that its point of attachment to the cord shifts outwards somewhat: that the distal portion of the process enlarges considerably, and becomes a spinal ganglion: finally, that these processes originally consist throughout of spherical nucleated cells, differing widely in appearance from the adjacent mesoblast-cells; but that, in the course of development, many of these cells become elongated and fibrillar, and that the distinction between the cells of the nerve and the mesoblast-cells becomes much less evident.

Hitherto all accounts of the development of the nerves in the chick, with the single exception of that given by His, agree in stating that the nerves, both cranial and spinal, arise in the mesoblast, and acquire their connection with the neural canal by a subsequent growth inwards[1].

His however has given a very different account[2], which may be briefly summarized thus:—according to him the first stage in the development of the posterior roots consists in the appearance of downgrowths of the external epiblast on either side of the summit of the spinal cord: these downgrowths are more strongly marked at intervals corresponding in number to the muscle-plates: they then separate from the external epiblast and form groups of cells, triangular in transverse section, situated between the spinal cord on the inner side, the protovertebra on the outer side, and the external epiblast above. These groups of cells develope into the spinal ganglia: they develope processes inwards to join the spinal cord, and outwards to form the part of the nerves beyond the ganglia.

The above description agrees with mine, and differs from the usual accounts (1) in assigning an epiblastic instead of a mesoblastic origin to the nerves: (2) in describing the nerves of the body as arising perfectly independently of the protovertebræ, instead of from parts of them. We also agree in describing the cranial nerves as arising in the same manner as the posterior roots of the spinal nerves.

My observations however lead me to differ from His on the following points:—(1) I find the nerves arise as outgrowths from the neural canal instead of from the external epiblast; (2) I do not find the ganglion to be the first part developed. Other differences, such as the development of the continuous longitudinal ridge, of which His omits all notice, readily suggest themselves: but the two just mentioned seem to me to be the most fundamental.

I have found that opposite the centre of each protovertebra the external epiblast does really grow downwards as a conical process on either side of, and in close contact with, the neural canal. From comparison with His' figures and descriptions,

[1] Foster and Balfour, *Elements of Embryology*, Pt. I. pp. 151, 152.
[2] *Die erste Anlage des Wirbelthierleibes.* Leipzig, 1868.

there is no doubt that these processes are the same that he describes: they are well marked in the body, especially in its hinder part, but are only very slightly developed in the head: they are of very slight longitudinal extent, and differ widely in appearance from the nerve-roots, with which I am perfectly satisfied they have no connection whatever, except that of simple apposition.

His gives several figures showing different stages in the development of the nerves, both cranial and spinal. From a careful examination of these figures and the descriptions given by His and by comparing them with my own specimens, I am convinced that His really saw the early stages of development, but was led into error as to their nature by imperfectly prepared specimens. He makes no attempt to represent the histological details of his sections, which we have seen to be of great importance in studying the early stages.

His figures two sections[1] through the hind-brain of a 38-hours chick, in both of which there is a large mass of cells on the summit of the brain between it and the external epiblast. The figures, it is true, show no connection between this mass and the brain, but neither do they show any between the mass and the external epiblast. The mass in fig. 3 corresponds exceedingly closely in appearance and position with the outgrowth (*m*), fig. 3, Pl. v. and is unquestionably the same structure. The mass in the other section, which is described as passing through the mid-brain, but which I believe to pass through the anterior part of the hind-brain, is somewhat smaller, and closely resembles that which I have figured in Pl. v. fig. 1.

Though therefore I differ from His on several fundamental points, I can appeal confidently to these two figures as confirming my statements, as far as the position and general appearance of the outgrowths are concerned, at the period mentioned.

His commenced by studying the spinal nerves, where he was misled by the downgrowths of the external epiblast. Had he commenced with the cranial nerves he could hardly have fallen

[1] *Op. cit.* Taf. VII. fig. 2, 3.

into this mistake, as, according to his own figures, the down-growths of epiblast in the head are very slightly developed.

It is important to notice that His suggests that the portion of nerve connecting the spinal ganglion (which he wrongly supposes to be the earliest developed part of the nerve) with the cord may possibly be an outgrowth from the cord, and not from the ganglion: he decides however in favour of the latter view.

Though the results of my observations thus differ widely from any previously published account of the chick with which I am acquainted, they will be found to agree remarkably closely with the account given by Balfour[1] of the development of the nerves in Elasmobranchs, which they serve to confirm in several of the most important points. This agreement refers not only to the general features, but even to the minute histological details. I have already had occasion to notice some of the points of this agreement: but in order to appreciate it fully, it is necessary to compare his figures and descriptions with those given here. Since an account of Balfour's researches, which have been extended considerably since the publication of the paper above referred to, appears in the present number of this Journal, I may refer the reader to his own paper for the detailed description.

Hensen[2] has described and figured the posterior roots of the spinal nerves as arising in the rabbit as direct outgrowths from the summit of the spinal cord: his figures correspond fairly closely with that given in Plate V. fig. 7. As regards the chick however, his own observations, strangely enough, lead him to adopt the same view as His, viz. that they are developed from the deeper layer of the external epiblast.

A mode of development of the nerves that is common to a group of vertebrates with very generalized affinities, Elasmobranchs (Balfour), and to two highly specialized groups, Mammals (Hensen) and Birds, may fairly be assumed to be typical of the sub-kingdom, and will probably prove to be the actual mode of development in other vertebrate groups besides those mentioned[3].

[1] *Op. cit.*

[2] *Zeitschrift f. Anatomie u. Entwickelungsgeschichte*, 1876. Bd. 1.

[3] Since writing the above I have satisfied myself that the description just given of the development of the nerves in the chick will apply also to the cranial

I have not yet determined the exact date of the earliest appearance of the nerves: in a chick of 43 hours we have seen the rudiments of the most important cranial nerves already established, as well as the posterior roots of the first six or more spinal nerves. I have not yet made satisfactory preparations of younger specimens showing the nerves, but judging from the rate of growth afterwards, we cannot be far wrong in assigning a period a little before the middle of the second day as the time of the first appearance of the nerve-rudiments.

The spinal nerves are developed in succession from before backwards: while, judging from the figures given by His, the cranial nerves appear first of all.

The anterior roots of the spinal nerves are not easy to investigate. My observations on their development are not so complete as I could wish, but, as far as they go, accord fairly well with those of Balfour on the Elasmobranchs.

They appear later than the posterior roots, a fact recognized by His, arising as outgrowths from the lower part of the sides of the spinal cord.

In Plate V. fig. 8, it will be noticed that the superficial cells of the lower part of the cord converge slightly towards a point (*a*); this is the spot at which the anterior root will shortly be developed; and this convergence is usually recognizable a short time before the actual appearance of the root.

The next stage is shown in Plate VI. fig. 10, a transverse section though the cervical region of a 4-day duck embryo, which shows the anterior roots as small processes projecting outwards from the spinal cord. The section is taken only a short distance behind the head, a region in which, owing to the mesoblast being less dense than it is further back, the early condition of the nerves is comparatively easy to study. Further back the mesoblast is very compact, vide Plate V. fig. 8, and the anterior roots difficult to recognize, especially when small.

The outgrowth to form the anterior root (*a*) is very slender

nerves of the Tadpole; and, I have strong reasons for adding, to the nerves, both cranial and spinal, of the Salmon. My observations in Salmon-embryos are, however, very imperfect as yet.

at its origin, and is at first directed outwards and somewhat upwards: it then turns downwards at an open angle, at the same time enlarging somewhat, though still remaining much more slender than the posterior root. It consists from the first of elongated fusiform cells, except at the attachment to the cord, where some of the cells are small and spherical.

Horizontal sections show that each anterior root at a very early stage consists of a series of small outgrowths placed one in front of another and converging slightly as they pass outwards into the mesoblast. Each anterior root has, about the 75th hour, a longitudinal extension equal to about half a protovertebra, opposite the anterior half of which it is at first situated; the posterior roots being at this period about opposite the intervals between successive protovertebræ. I have not been able to determine whether each anterior root arises originally as a single outgrowth, or whether it consists from the first of a series of outgrowths. These stages are difficult to investigate owing to the exceedingly slender attachment of the anterior roots to the spinal cord. The origin of the anterior roots by several processes is a point in which the chick differs from Elasmobranchs.

In Plate VI. fig. 10, the posterior roots (*p*) are seen in section, but not their points of attachment to the cord.

The last stage with regard to the spinal nerves which I propose to consider in the present paper is shown in Plate VI. fig. 9, which represents a transverse section through the anterior dorsal region of a 75-hour chick, passing through both anterior and posterior roots. The anterior have grown out to meet the posterior roots and so form the spinal nerves, which run downwards on the inner side of the muscle-plate.

The posterior root is seen to be attached on the left side by a small pedicle to the upper part of the side of the cord: on the right side the section has just missed the point of attachment. It will be noticed that this point of attachment of the posterior root is considerably lower than it was in Plate V. figs. 7 and 8: a more important difference is that the nerve-root is no longer attached by its extremity to the cord, but forms a considerable swelling above this point of attachment.

We have already seen a tendency on the part of the roots to shift their attachments outwards: this is shown in fig. 7, and has been explained as due to rapid growth of the cells at the summit of the cord. This shifting may have increased so much as to cause the marked change in position seen in fig. 9; in which case the growth upwards of the nerve above the root would be a secondary change.

Another possible explanation is that the original attachment to the top of the cord has been completely lost, and a new one developed in the situation of the permanent posterior root: in this case the outgrowth above the point of attachment would simply be the remnant of the original root.

My preparations have not enabled me as yet to determine with certainty which of these explanations is the correct one; but the following facts seem to speak strongly in favour of the latter one. During the stage represented in fig. 8, it is exceedingly difficult to determine whether the posterior root is still attached to the cord or not; and it is only in occasional sections that I have been able to trace the connection. When the connection was demonstrated, it was, as shown in fig. 8, exceedingly slender. I have never observed specimens in which the nerve was attached at a point lower than that shown in fig. 7, yet above that in fig. 8. The outgrowth above the point of attachment rapidly becomes smaller, and is lost in specimens but little older than that in fig. 9: this transitory nature is readily comprehensible on the one hypothesis, but a serious difficulty on the other; as it would be very hard to understand why such an outgrowth should arise at such a stage in development, and disappear again so rapidly. The general lines of direction of the cells composing the cord, which are indicated in fig. 9, strongly favour the view that the permanent attachment is a secondary one, and not the original one altered in position; while at the same time they favour the view which regards the outgrowth above the point of attachment as a remnant of the primary root. Lastly, the cells composing this projection differ from the cells of which the rest of the root consists in being spherical or nearly so, while the rest of the root is made up of considerably elongated cells. This rounder form causes them to resemble the cells of the original out-

growth. However, as I have not yet traced all the stages, I cannot consider the point as settled.

The outgrowth in question has been observed in Elasmobranchs by Balfour, who describes it thus[1]: "the proximal portion (of the nerve-rudiment) presents a fairly uniform diameter, and ends dorsally in a rounded expansion: it is attached, remarkably enough, not by its extremity, but by its side, to the spinal cord." He however does not adopt the suggestion here made as to its origin and meaning, but considers it to be part of a dorsal longitudinal commissure he has detected connecting the posterior spinal roots together. I have failed to detect this commissure in the chick. Balfour's figures H. 1. and I. 1. correspond closely, as far as this outgrowth is concerned, with Pl. VI. fig. 9.

The ganglion (*g*) is but slightly developed at this stage. The attachment of the anterior root to the cord is seen to be still very slender: the convergence of the cells of the cord to this point—indicated in the figure by the lines on the cord—is very well marked. Outside the cord the anterior root first passes outwards and upwards for a very short distance, dilating as it does so; it then bends rather sharply downwards, and, becoming considerably thicker, joins the posterior root and runs down on its inner side. The two roots can be readily distinguished from one another owing to the cells of the anterior root being very much more elongated than those of the posterior. The two roots run side by side for a short distance without blending, but further on become completely fused.

My study of the cranial nerves has been confined as yet entirely to the earlier stages; and even with regard to them my observations are very fragmentary and imperfect. Still some points of interest have presented themselves, to which I shall refer briefly.

We have seen that, towards the end of the second day, there is behind the auditory pit a continuous outgrowth from the summit of the hind-brain, of considerable longitudinal extent. This outgrowth is connected—by means of the longi-

[1] *Op. cit.* p. 185.

tudinal ridge so often alluded to—anteriorly with that from which the 7th and 8th nerves are derived, and posteriorly with that which gives origin to the posterior roots of the first pair of spinal nerves. From it the vagus and glossopharyngeal nerves are derived.

Though I have not worked out the later stages satisfactorily, the following points suggest themselves as worthy of notice. Firstly, the marked tendency of the vagus outgrowth to pass outside the muscle-plate between it and the superficial epiblast: this has been already noticed. Secondly, the fact that transverse sections through the hinder part of the vagus outgrowth pass also through the first protovertebra, vide fig. 5: in other words, that the vagus outgrowth, which is of considerable longitudinal extent, reaches backwards so as to overlap the anterior half of the first protovertebra. Thirdly, I would note as a point of some morphological interest the fact that the glossopharyngeal and the whole of the vagus arise at first as a *single continuous outgrowth*[1], from the distal edge of which the several branches are subsequently derived. If then the vagus is to be considered as equivalent to a number of spinal nerves fused together—a view in favour of which there is a considerable amount of evidence—this earliest condition of the vagus outgrowth may prove to be an indication that the fusion first occurred at a very early period in the phylogeny of the chick, and possibly in that of other vertebrates also.

I have no observations on the development of the spinal accessory and hypoglossal nerves; but the backward extension of the vagus outgrowth over the anterior protovertebra may help to render it intelligible how one or other of these nerves may appear in one group of vertebrates to be cranial, in another spinal.

Pl. VI. fig. 12, represents a longitudinal section through the head and neck of a 4-day duck embryo; passing through the mid-brain (*mb*), hind-brain (*hb*), and anterior part of the spinal cord (*s*). The section is slightly oblique, passing rather deeper on the left than the right side. On the right side one of the branches (*v*) of the vagus outgrowth is seen cut transversely; on the left side at (*v'*) the section passes through the point of origin

[1] Vide also Foster and Balfour, *Elements of Embryology*, Part I. p. 138.

of the vagus outgrowth: at (*v*) on the same side a part of the vagus is seen in the form of a longitudinal rod; but whether this corresponds to the commissure described by Balfour[1] as connecting all the roots of the vagus in Elasmobranchs, I have not determined. At *p*. are seen sections of the posterior roots of a spinal nerve.

Immediately in front of the auditory involution a single large root arises on each side, from which both the auditory and facial nerves are derived. This is represented in transverse section in Pl. V. fig. 3; and in horizontal section in Pl. VI. fig. 11, which is a horizontal section through the neck and hind-brain of a 75-hours chick. A large nerve, (*b*) fig. 11, is seen arising on each side from the second of the dilatations of which the hind-brain consists: it runs backwards and expands considerably, forming a large mass closely applied to the anterior wall of the auditory vesicle (*aud*).

In Pl. VI. fig. 12, the facial nerve (*f*)—which is derived from the anterior part of the outgrowth common to it and the auditory nerve—is seen passing down in front of the auditory vesicle, from which it is quite distinct. On the right side of the section, which, as just noticed, is at a deeper level than the left, and passes below the auditory vesicle, the facial nerve (*f*) is still seen, but lies somewhat further back than it did on the left side, showing that it grows at first downwards and slightly backwards.

The 5th nerve has already been seen in transverse section in Pl. V. fig. 4, arising as an outgrowth of very slight vertical thickness from the summit of the anterior dilatation of the hind-brain. In Pl. VI. fig. 12 *t*, it is shown in horizontal section at a somewhat later stage.

The root is seen to have a considerable longitudinal extension now; and transverse sections at this period show that the vertical extension has also increased. The point of attachment has shifted down from the extreme summit of the brain, and is situated some distance lower down. The nerve runs outwards, increasing considerably in width, and divides distally into two branches, an anterior smaller and a posterior larger one. These

[1] A Preliminary account of the Development of Elasmobranch Fishes. *Quart. Journ. Micros. Science*, 1874. Plate XV. fig. 14. *v.g.*

two branches I have been able to identify with the two described by Foster and Balfour as existing at the end of the third day[1]. Though the 5th nerve arises as a single outgrowth on either side, yet the condition of the vagus and glossopharyngeal in their earliest stages must render us very cautious about inferring that it therefore corresponds to a single spinal nerve.

Figs. 11 and 12 show that the so-called "hind-brain" does not consist of a single vesicle, but of a series of dilatations, separated by slight constrictions, and gradually decreasing in size from before backwards. Of these the most anterior and largest one, which at the end of the second day is but little smaller than the mid-brain, gives origin at its widest part to the 5th nerve. This relation I have found to occur invariably in all embryos up to the end of the fourth day that show any trace of a 5th nerve: it confers a considerable amount of constancy on this dilatation[2].

From the second dilatation the combined root of the 7th and 8th nerves arises. I am not however satisfied that this relation is invariable. The succeeding dilatations are much smaller and closer together, and do not appear to be constant in number or relations.

Not only does the whole "hind-brain" consist of a series of these dilatations, but the spinal cord also presents a similar, though less strongly marked, series; being slightly constricted opposite the centre of each protovertebra, and dilated opposite the intervals between successive pairs.

At the 50th hour a small outgrowth from the mid-brain is visible on either side close to the median dorsal line; this grows rapidly, and by the end of the fourth day forms a nerve of considerable size running from the mid-brain to the posterior part of the eye, and lying at a rather deeper level than the anterior (or ophthalmic) branch of the 5th nerve, which it crosses almost at right angles. From its position and relation it can

[1] *Elements of Embryology*, Part I. pp. 137, 8, and fig. 40, p. 142.

[2] Foster and Balfour (*loc. cit*, p. 138) mention the series of dilatations as existing on the third day, and suggest that they "may perhaps be viewed as indications of an aborted segmentation of the hind-brain into a series of vesicles." It is therefore a matter of some importance to determine whether they possess any constancy in their relations.

only be the 3rd nerve, which is thus from the first perfectly independent of the 5th.

My observations on the development of the olfactory nerves have led to results which differ materially from the ordinarily received accounts. According to Foster and Balfour[1] an "olfactory vesicle" grows out from the under surface of the cerebral hemisphere of either side towards the end of the third day; while the superficial epiblast is driven in to form a nasal pit. The pit and vesicle are not connected at first. This connection is generally described as brought about by the development of an olfactory nerve in the mesoblast, between the vesicle and pit. My own observations lead me to the conclusion that the olfactory nerves really arise as solid outgrowths from the anterior part of the fore-brain, near the median dorsal line.

Pl. VI. fig. 13, represents about half of a section taken through the fore part of the head of a 4-day duck-embryo in a plane transverse to the longitudinal axis of the fore-brain. The figure is semi-diagrammatic, the mesoblast being entirely omitted and no attempt made to represent the histological details. The section passes through the olfactory depressions, of which that on one side only is shown. The external epiblast is seen to be very thin over the roof and floor of the fore-brain, but is thickened at the sides, and driven in so as to form a shallow pit—the olfactory pit or involution (*na*)—of which the thickened epiblastic lining will become the special olfactory epithelium. The fore-brain is approximately circular in section; its walls are rather thinner at the top and bottom than at the sides. The olfactory nerve (*olf*) is a short solid body stretching from the upper part of the fore-brain downwards and outwards to the upper part of the olfactory pit: it consists of elongated fusiform cells which are in intimate relation on the one hand with the walls of the fore-brain, and with the cells of the olfactory epithelium on the other. The actual connection of nerve and brain was not seen in the section figured, but in one a little further back.

The same structures are shown in another plane in Pl. VI. fig. 14, which represents a horizontal section through the fore part of the head of a 75-hours chick, in a plane parallel to the

[1] *Loc. cit.* p. 117.

longitudinal axis of the fore-brain. If it is borne in mind that figs. 13 and 14 represent sections of the same parts taken in planes at right angles to one another, the relation of the parts will be readily understood. It will be seen that the section in fig. 14—which is not perfectly horizontal—passes through the olfactory pit on the left side, and on the right side just above it, so as to miss the pit, but cut the olfactory nerve (*olf*). The nerve is seen at this point to be quite distinct from the brain.

Fig. 15 is a section from the same embryo as fig. 14 and parallel to it, but in a slightly higher plane. On the left side the nerve has approached somewhat nearer the middle line, and lies very close to the brain, from which however it is still perfectly distinct. On the right side the section passes through the point at which the nerve is attached to the brain.

The condition of the fore-brain requires some notice, as I have found the ordinary accounts to be somewhat misleading. Fig. 14 shows that the fore-brain at 75 hours is considerably dilated in front of the optic vesicles, forming a large prominent swelling, which occupies the extreme front of the head, and is nearly circular in transverse section (fig. 13). The cerebral hemispheres appear first as dilatations of the sides and upper part of this expanded fore-brain, and when seen in horizontal section bear a very similar relation to the fore-brain that the commencing optic vesicles originally did (vide Pl. VI. fig. 15 *ch*).

Of an olfactory vesicle there is no trace whatever in the early stages. The olfactory nerves at first arise from the fore-brain, and not from the cerebral hemispheres: this is shown very clearly in figs. 13, 14 and 15, from which we also see how the subsequent growth forwards of the cerebral hemispheres will cause the nerves to appear to spring from their under surface.

The appearance, position and relations of the olfactory nerves at the 75th hour so closely resemble those of the other cranial nerves described above, as to strongly suggest that they are strictly comparable; and that the olfactory nerves are really the first pair of true cranial nerves. More accurate observations than we appear to possess at present on the development of the olfactory nerves in less specialized vertebrates are, however, necessary before this point can be considered as established.

If the olfactory nerves really prove to be, as I have just suggested, the first pair of true cranial nerves, many of the theories propounded concerning the composition of the vertebrate head will require modification. I will only allude here to the value attached to the distribution of the cranial nerves by Prof. Huxley, who starts with the assumption that the 5th nerve is the most anterior of the true cranial nerves.

In conclusion, I would say a few words on the distinction between the head and body. Sections of the neural canal, whether transverse or horizontal, do not enable us to fix any point as a limit between brain and spinal cord. In different specimens the sections vary considerably in appearance: in some the characteristic oval section of the cord—as seen in Pl. v. fig. 6—is attained in parts that are unquestionably brain, while in others it is not acquired till the second protovertebra.

We have also seen that the outgrowths of cells from the summits of the brain and cord are perfectly continuous, and present no character that enables us to mark a limit between head and body.

The vagus-root has already been alluded to as overlapping the anterior half of the first protovertebra; and the close similarity in form between the vagus outgrowth and that for the spinal nerves has been pointed out.

Since then no definite indication of a limit between head and body is afforded by either the neural canal, the longitudinal outgrowths from its summit, or by the mode of development of the nerves, we must conclude that all these features were acquired before the distinction between head and body.

At the end of the second day the only means of fixing a limit is furnished by the protovertebræ; the anterior border of the first protovertebra marks the posterior border of the head. Here however we must bear in mind the fact that the anterior protovertebra is not the first to be developed[1].

Under these circumstances Balfour's failure to detect anterior roots to the cranial nerves of Elasmobranchs[2] becomes of special importance, as indicating the possible existence of a sharp distinction between head and body. I have not yet

[1] Foster and Balfour, *loc. cit.* p. 57.
[2] *Loc. cit.* p. 189, note.

detected anterior roots in the Bird in any sections taken in front of the first protovertebra; but, owing to the vagus-root overlapping the first protovertebra, I am by no means certain that the most forwardly situated of the anterior roots does not really belong to the vagus. Since however I have not examined embryos later than the end of the fourth day, and have not identified several of the cranial nerves at all, I cannot attach much importance to this failure, nor consider it as affording any decided confirmation of Balfour's observations just alluded to.

EXPLANATION OF THE FIGURES.

All the drawings were outlined with a Hartnack camera: the objective indicated as employed in each case is merely that used in drawing the outline: the details were filled in from a Hartnack obj. 8; oc. 3, and Zeiss obj. F; oc. 3.

Plate V.

ep. external epiblast. *hb.* hind-brain.
m. outgrowing mass from top of neural canal.
aud. auditory pit. *n.* notochord.
s. spinal cord. *mp.* muscle-plate.
g. spinal ganglion.
a. position of anterior root of spinal nerve.

Figs. 1—6. Represent transverse sections from the same embryo—a 43-hours chick. Picric acid. Hartnack camera obj. 4.

Fig. 1. Section through hind-brain, passing through deepest portion of auditory pit (*aud*).

Fig. 2. Through hind-brain, a short distance in front of fig. 1.

Fig. 3. A short distance in front of fig. 2: passes through common root (*m*) of 7th and 8th nerves.

Fig. 4. Section through the most anterior dilatation of the hind-brain, passing also through the 5th nerve (*m*).

Fig. 5. Section taken a short distance behind fig. 1; passing through the anterior part of the first protovertebra—(*mp*): and through the vagus-root (*m*).

Fig. 6. Through posterior part of first protovertebra: passing also through the first spinal nerve (*m*).

Fig. 7. Transverse section through the dorsal region of a 3-day chick. Hartnack camera obj. 4. Shows posterior spinal roots (*m*), and points of attachment to cord.

Fig. 8. Transverse section through the posterior dorsal region of a 4-day chick embryo. Hartnack camera obj. 4. Shows spinal ganglion (*g*) and point (*a*) at which the anterior root will shortly appear.

Plate VI.

All the figures in this plate are semi-diagrammatical; no attempt having been made to represent the mesoblast or the histological details.

Fig 9. Transverse section through the anterior dorsal region of a 75-hours chick, passing through both anterior and posterior spinal roots. Hartnack camera obj. 4.

s. spinal cord. *mp*. muscle-plate.
p. point of attachment of posterior root to cord.
g. ganglion. *a*. anterior spinal root.
n. notochord.

Fig. 10. Transverse section through the cervical region of a 4-day duck embryo, taken just behind the head. Hartnack camera obj. 2.

a. anterior spinal root. *p*. posterior spinal root.
n. notochord. *mp*. muscle-plate.

Fig. 11. Horizontal section through the hind-brain of a 75-hours chick. Hartnack camera obj. 2, reduced ⅘.

hb. cavity of hind-brain. *aud*. auditory vesicle.
b. root of 7th and 8th nerves. *n*. notochord.

Fig. 12. Horizontal section through head and neck of a 4-day duck embryo. Hartnack camera obj. 2, reduced ½.

mb. cavity of mid-brain. *hb*. cavity of hind-brain.
s. spinal cord. *mp*. muscle-plate.
t. root of 5th nerve. *f*. facial nerve.
aud. auditory vesicle. *v*. vagus.
v′. root of vagus. *p*. posterior roots of spinal nerves.

Fig. 13. Section through fore part of head of a 4-day duck embryo, in a plane transverse to the longitudinal axis of the fore-brain. Hartnack camera obj. 2.

ep. external epiblast. *fb*. cavity of fore-brain.
na. nasal pit. *olf*. olfactory nerve.

Fig. 14. Horizontal section through fore part of head of a 75-hours chick embryo. Hartnack camera obj. 2, reduced ½.

fb. cavity of fore-brain. *na*. nasal pit.
olf. olfactory nerve.

Fig. 15. Section from same embryo as fig. 14, and parallel to it, but in a slightly higher plane. Hartnack camera obj. 2, reduced ½.

fb. cavity of fore-brain. *ch*. cerebral hemisphere.
olf. olfactory nerve. *ep*. external epiblast.

From the *Journ. of Anat. and Phys.*, Vol. XI.]

THE CRANIAL OSTEOLOGY OF AMIA CALVA. By T. W. BRIDGE, B.A., *Trinity College, Demonstrator of Zoology and Comparative Anatomy in the University of Cambridge.* (Pl. VII.)

(From the Zoological Museum.)

THE cranial osteology of living Ganoids has been hitherto but partially investigated; and even those genera that have been described by the older anatomical writers will abundantly repay renewed investigation now that the researches of Parker, Gegenbaur, and Huxley, have thrown so much light upon the morphology of the Vertebrate skull.

Agassiz[1], it is true, has given to us an elaborate account of Lepidosteus, and the earlier description of Polypterus by H. Müller[2] has been supplemented by Dr Traquair's[3] opportune paper; while to Dr Günther[4], and Prof. Huxley[5], we are indebted for exhaustive accounts of the skeleton of Ceratodus.

On the other hand, I am not aware that, beyond the more or less brief accounts to be found in Joh. Müller's *Vergleichende Anatomie der Myxinoiden*[6], we have any detailed descriptions of Spatularia, Acipenser or Amia; and the anatomical student who may wish to acquire any complete knowledge of these genera must content himself with the above-mentioned references, or with such facts as he may be able to glean from such text-books as Huxley's *Manual of Vertebrata,* Owen's *Comparative Anatomy,* or the *Grundzüge der Vergleichenden Anatomie* of Gegenbaur.

More especially is this true of Amia. The zoological characters of this genus have been described by several zoologists. Vogt[7] first detected its true position among the Ganoids, and removed it from the Clupeoid Teleostei with which it had been placed by Müller[8]; and Hyrtl[9] and Franque[10] have described

[1] Agassiz, *Poiss. Foss.* Tom. II.
[2] *Abhandl. Ak. Wiss. Berlin*, 1844.
[3] *Journal of Anatomy*, Vol. IV.
[4] *Phil. Trans.* 1871.
[5] *Proc. Zool. Soc.* 1876.
[6] *Vergl. Anat. d. Myx. Berlin*, 1835.
[7] *Annales des Sciences Naturelles*, Tom XXIV. Heart and Alimentary Canal figured.
[8] Müller's paper "Sur les Ganoïdes et sur la classification naturelle des Poissons" is translated by Vogt in the XXV. Vol. *Ann. Sc. Nat.*
[9] *Ak. Wiss. Wien*, 1855.
[10] *Amiae calvae Anatomia*, Berlin 1847.

the generative organs and visceral anatomy. But, I am not aware that there exists any connected account of the osteology of the skull of this genus, or that the skull has been figured. As I have lately had the opportunity of dissecting a full sized specimen of Amia calva, and as several interesting facts were elucidated which I have never seen mentioned in any of the text books, or anatomical memoirs on the Ganoids, I have ventured to give the following description of its skull.

Ganoid plates and membrane bones of the cranium. (Figs. 1 and 2.)

The flattened and depressed cranium is invested externally by a series of ganoid plates, all of which are firmly attached to the underlying cartilage by means of the osseous lamina adhering to the under surface of each ganoid plate. The external highly polished surfaces of the plates, with the exception of the nasals and the dermo-ethmoid, are destitute of any covering of soft skin, and all of them have their surfaces sculptured into wavy and branching rugosities, which radiate from the centre towards the circumference of each plate.

Viewed from above (fig. 1) the following bones are seen. Overlying the occipital region there is, as in many of the Siluroidei, a large square dermo-supraoccipital (*d.so*). This bone rests upon the subjacent cartilage of the occipital region of the chondrocranium, and by its hinder margin upon the epiotics; laterally it articulates with the two parietals, in front with the hinder edges of the frontals, while posteriorly it is in contact with the supra-temporals. Owen[1] says that this bone is divided by a median suture, but in my specimen there was no trace of such a suture. The dermo-supraoccipital is flanked on either side by a bone which overlies the pterotic region of the otic capsule, and occupies much the same position in Amia as the bone marked "Pa. Ep." in Huxley's[2] figures of Clarias, and the bone usually called dermo-epiotic in many Siluroids. I shall call this bone parietal, though I am not sure that it would not be better to call it 'dermo-pterotic,' or 'dermo-epiotic.'

[1] *Comparative Anatomy*, Vol. I.
[2] Essay on the Classification of the Devonian Fishes, *Mem. Geol. Survey*, Dec. 10. Figs. 21, 22.

Each parietal (*pa*) is triangular in shape, with straight external and posterior margins, but articulating by a sinuous internal edge with the dermo-supraoccipital and frontal bones. Its base is in contact with the supra-temporal, and its apex with the dermo-sphenotic. Each parietal, like most of the other investing ganoid plates, has its under surface much thickened by an adherent parostosis, which, along the outer margin of the plate, is produced downwards into a short vertical ridge, which rests upon the pterotic region of the subjacent cartilage, and upon the opisthotic. This vertical plate prevents the horizontal portion of the parietal from resting directly upon the cartilage, and consequently, when the skull is viewed from behind, a vacuity is to be seen bounded by the horizontal and descending plates of the parietal, the epiotic and the cartilage of the cranial roof. The outer margin of each parietal fulfils the function of a supra-temporal in transmitting the cephalic continuation of the main lateral slime-canal.

The two supra-temporals (*s.tp*) are triangular bones with their apices pointing inwards towards each other, but not coming into contact; behind they overlap the post-temporals (*p.tp*). Each is pierced by the main lateral slime-canal in its passage forwards to the parietal, and, in addition, each is traversed by the transverse canal by which the two lateral canals of opposite sides are connected with each other. In Polypterus these two bones are represented by a chain of six transversely disposed ossicles, through each of which the transverse slime-canal passes, and a similar arrangement is found in Lepidosteus.

The frontals (*fr*) are the largest of the investing cranial bones; they cover the greater part of the cranial roof, extending from the sphenotics behind to the prefrontals in front, and as their width is much greater than that of the interorbital region of the cranium, they roof over the orbits. The frontals articulate with each other in the median line by an interdigitating suture; behind, they are in contact with the parietals and dermo-supraoccipital; and in front they articulate by a straight suture with the posterior margins of the nasals.

Small dermo-sphenotics (*pt.f*) overlie the true sphenotics and lie along the outer margin of each frontal; their anterior edges mark the posterior limits of the orbits. At the anterior and

external edge of each frontal there is a small dermo-prefrontal overlying the true prefrontal.

All the bones that have yet been described are obviously composed of an external ganoid plate, and of an internal and much thicker lamina resulting from the ossification of the subcutaneous tissue; but the ganoid plates, to be presently described, appear to be composed of the ganoid element only. The large paired bones (*na*) in front of the frontals, directly overlie the nasal capsules, and by their emarginate anterior edges bound the foramen for the anterior nares (*a.n*). These bones, at first sight, greatly resemble the paired dermo-ethmoids of Polypterus, but as the T-shaped bone in front of them appears to be the true dermo-ethmoid, I shall call these paired bones, nasals.

The outer margin of each nasal is in contact throughout its whole length with a long and slender præorbital bone (*p.orb*). There is a bone occupying much the same position in Clarias, and in epicrium among Amphibians.

The dermo-ethmoid (*eth*) is somewhat T-shaped, with its anterior transverse part slightly concave from side to side. It overlies the prenasal process and the premaxillæ. Each end of the transverse part is in contact with the præorbital bone, while the stem of the T passes backwards between the nasals, separating them for about a third of their extent.

The orbit is bounded in front, below, and behind by a series of five orbital bones; as there are no supra-orbital bones the orbit is limited above by the frontal. A large lachrymal plate (*l*) bounds the orbit in front, and two very large wedge-shaped post-orbital bones (*pt.orb*), which extend backwards over the cheeks bound it posteriorly. These bones appear to be represented in Polypterus by a single large cheek-plate (marked *Y* in Dr Traquair's paper), which appears to have coalesced behind with the præoperculum. The lachrymal and postorbital bones are connected beneath the orbit by two small suborbital pieces (*s.orb*). All the orbital ganoid plates have their orbital margins much thickened by subcutaneous ossification.

Opercular bones. (Fig. 2.) As in the typical Teleostei there are four opercular bones. The preoperculum (*p.op*) is a narrow crescentic bone firmly anchylosed to the hyo-

mandibular and symplectic bones. The mandibular branch of the great lateral slime-canal traverses the whole length of the preoperculum, and gives off several short transverse branches in its course. There is a large operculum (*op*) articulating with the condyle on the hyomandibular, a small interoperculum (*i.op*) connected by ligament with the angle of the mandible, and, wedged in between the two last-mentioned elements—there is a suboperculum (*s.op*).

In this specimen of Amia there were eleven branchiostegal rays. They increased in size progressively, and the uppermost one, which is the largest, is attached by a special articulation to a facet on the outer side of the epihyal. The rays are all attached to one side—the outer side—of the ceratohyal, and not to both sides as is commonly the case in Teleostei.

In comparing the skull of Amia with the skulls of certain of the Siluroidei, and notably with that of Clarias, it is interesting to notice that, in addition to the more obvious and less important points of resemblance between the two genera necessitated by the flattened condition of the head, and a foreshortening of the prefrontal region, there is a close agreement between them in the number and relations of their ganoid plates.

In Clarias, as in Amia, there is a median dermo-supraoccipital; there are also 'parietals' or dermo-epiotics, dermosphenotics, and dermo-prefrontals; paired frontals and nasals; and a median T-shaped dermo-ethmoid. The ethmoid is proportionally smaller, and the nasal proportionally larger in Amia than in Clarias, so that the ethmoid completely separates the nasals in the latter fish; but otherwise there is but little difference between the two forms in the disposition of their ganoid plates.

In Clarias there are also two large postorbital bones covering the cheeks and extending nearly to the preoperculum. This genus has supra-temporals and preorbitals, as in Amia.

The ventral surface of the cranium is invested by a parasphenoid and vomers.

The former bone (*pa.s*) extends along nearly the whole length of the base of the cranium, from the vomers in front to

the posterior margin of the basioccipital. Behind, this bone is somewhat spoon-shaped, and in front it partially separates the two vomers from each other. About the middle of its length the parasphenoid gives off two lateral wings (*a.p*), one on each side, which curve upwards in front of the prootic and between the foramina for the exits of the fifth and seventh nerves till they abut against the post-frontals. From the base of each lateral ala a small process is given off which unites with the descending process of the alisphenoid, and so forms the outer boundary of the canal through which the muscles of the eyeball pass.

The upper surface of the parasphenoid is marked by a longitudinal ridge, which is firmly adherent to the grooved inferior surface of the basi-occipital and the cartilaginous basis cranii. The lower surface of the bone is garnished with a number of closely set asperities.

The anterior third of each vomer (*vo*) is suturally united to its fellow; the posterior two-thirds are separated by the intervention of the parasphenoid. The vomers carry a number of closely set conical teeth arranged in a crescentic series parallel to those in the premaxillæ.

Premaxillæ and maxillæ. (Fig. 3.) Each premaxilla (*p.mx*) consists of an expanded and thickened marginal portion in which the long and curved teeth fringing the anterior margin of the gape are situated, and of an ascending portion which passes backwards beneath the nasals in contact with the subnasal cartilage and inter-nasal septum as far as the anterior edges of the frontals. The spoon-like upper surface of this ascending plate upon which the nasal capsules lie is perforated in the centre by an oval foramen for the passage of the olfactory nerve to the olfactory mucous membrane. In the median line between the two premaxillæ the prenasal process (*p.n*) becomes visible. Superiorly the premaxillæ are covered by the nasals and dermo-ethmoid.

The maxillæ (*mx*) are very Teleostean. Each bone carries a number of small, but sharp teeth, and each has its anterior end prolonged into an inwardly curved process which rests in a groove in the extreme anterior end of the palatine. The

maxillæ do not form any part of the orbital boundary. Upon the upper part of the hinder margin of each bone there is a jugal bone.

Chondrocranium and its Ossifications. (Figs. 2 and 3.)

When the investing parostoses and ganoid plates just described have been removed it is seen that the cartilaginous roof of the chondrocranium is complete, there being no trace of the fenestræ which exist in all the other Ganoids with the exception of Lepidosteus. The chondrocranium is very depressed and somewhat wedge-shaped, broad behind in the occipital region and tapering gradually to the prenasal process anteriorly. Neither the periotic nor the olfactory regions form very conspicuous lateral prominences. Internally the cranial cavity is broad behind, and with a gradual diminution in width and height is continued forwards between the orbits to a point between the prefrontals, where it is terminated by a lamina perpendicularis. On each side of this lamina there is a large foramen through which the olfactory nerves pass to the nasal capsules. Each nasal sac rests upon a broad subnasal lamina, and is separated from its fellow by the internasal prolongation of the lamina perpendicularis, which finally terminates in a short conical prenasal process. The capsules are not invested by even the rudiments of alinasal outgrowths.

The occipital plane is greatly inclined forwards. The large basioccipital bone (*bo*) probably represents, in addition to its own proper element, the centra of at least two of the most anterior vertebræ; that this is so is probable from the fact that two neural arches are attached to the hinder part of the bone. Two large exoccipitals (*ex.o*) uniting below with the basioccipital by a persistent suture, and separated from each other superiorly by a narrow interspace of cartilage, bound the foramen magnum. The outer margin of each bone is deeply cleft by the foramen (*Vg*) for the vagus nerve. There is no proper supraoccipital.

In the auditory capsule there are distinct epiotic, opisthotic, sphenotic, and prootic ossifications. The epiotics (*ep.o*) are small, triangular ossicles, occupying their normal position in

relation to the arch of the posterior vertical semicircular canal, and situated immediately over the exoccipitals.

The opisthotics (*op.o*) protect the postero-lateral angles of the cranium, and, in conjunction with the exoccipitals, bound the vagus foramen. A small projecting spur unites the opisthotic to the prootic. The prootic (*pr.o*) is the largest of the otic bones. Externally it is nearly circular in shape, but is deeply notched in front for the exit of the facial nerve, and behind it gives up a process which suturally unites with the spur from the opisthotic. Though the prootics do not develope the descending outgrowths so characteristic of Teleostei, yet they give off internal plates which, uniting with each other in the median line of the cranial floor, form a characteristic 'prootic bridge' (Fig. 5).

The sphenotic bones (post-frontals) (*pt.f*) occupy the antero-lateral angles of the otic capsules.

There is no pterotic bone. As in Polypterus the pterotic region of the auditory capsule is covered by the lateral margin of the parietal.

With the single exception of the prootics none of the otic bones are visible from the inside of the cranial cavity; and, with the exception of the slender connection of the opisthotic and prootic elements, the otic bones are separated from one another by wide interspaces of cartilage. The prootic alone is traversed by the particular semicircular canal with which it is in relation; the remaining otic bones lie entirely superficial to their respective canals which traverse the cartilage only.

In front of the lateral alæ of the parasphenoid, and between the foramina for the fifth and optic nerves, is the alisphenoid (*al.s*). This bone is almost circular in shape; from the middle of its outer surface a small spicule of bone is given off, which arches over the canal for the orbital muscles and abuts against the smaller of the two lateral processes of the parasphenoid.

This descending process bears a singular resemblance to the "descending process of the alisphenoid" so common among Mammalia. The alisphenoid is perforated by a foramen for the first division of the fifth nerve (*V′*), and a little below this

aperture there is a second foramen for the exit of the second and third divisions of the same nerve (V'' and V''').

The orbitosphenoids (*o.s.*) resemble the alisphenoids in size and in their nearly circular outline; they are thin above and support the descending cartilaginous "roof-plates," but are much thicker below, where they rest upon the thickened lateral edges of the coalesced trabeculæ. Viewed from the interior of the cranium their inferior edges are seen to approach closely to each other, though they remain separated by the cartilage which forms the floor of the trabecular groove. The hinder border of the bone is suturally connected with the alisphenoid, and is cleft nearly to its centre by a triangular fissure for the passage of the optic nerve (II). An examination of the interior of this part of the cranial cavity shows that the wide and shallow pituitary fossa is bounded behind by the "prootic bridge" (*Pro.*), beneath which the fossa is prolonged for some distance, while the anterior clinoid wall is cartilaginous. A strong fibrous membrane forms a floor to the fossa, and in addition extends between the alisphenoids and orbitosphenoids and the subjacent cartilage, surrounding the optic nerves, and filling up what would otherwise be a considerable vacuity in the cranial walls. Immediately in front of the fossa the cartilage of the cranial floor is perforated by two small foramina for the entrance of the carotid arteries.

Resting upon, and in part embedded in, the cartilaginous anterior clinoid wall there are two small osseous nodules (*b.s.*). Each ossicle is triangular in shape, with its broad end resting on the cartilage, from the ossification of which the bone is, apparently, in part formed, while the apex extends into the fibrous membrane which has been described as forming the floor of the pituitary fossa. From the position of these ossicles it is probable that ossification commenced in the fibrous membrane, and that subsequently it invaded the anterior clinoid wall. Although these ossicles have only a fibrous connection with the alisphenoids their position and relations point to their homology with the prepituitary portion of the basisphenoid of other Fishes. According to Mr Parker[1] the

[1] Development of the Salmon's skull, *Phil. Trans.*, 1872.

prepituitary element of the basisphenoid in the Salmon first appears as an ossification in the membranous septum behind the optic nerves. At first this ossification is quite distinct from the adjacent cartilage, but as ossification advances the cartilage is invaded and the exosteal rudiment becomes a true endosteal centre. Thus, from their position in relation to the "trabecular crest," and in their in part membranous origin, these ossicles in Amia agree with the basisphenoid of the young Salmon. The existence of paired ossicles is no objection to the homology suggested, as in the Ophidia the basisphenoid has a similarly double origin. If these ossicles are rightly determined as representing a basisphenoid, then Amia differs from all other living Ganoids, and agrees with the Teleostei in possessing a rudiment of that bone.

I may add, that a careful examination of a fresh skull of Lepidosteus failed to reveal the existence of similar structures in that ganoid. In Lepidosteus the cartilage which forms the anterior clinoid wall is produced upwards on each side between the foramina for the fifth and optic nerves, till it reaches the lower edge of the alisphenoids; but there are no ossifications either in the cartilage itself or in the fibrous floor of the fossa. In this fish, as in Amia, the posterior clinoid wall is formed by a "prootic bridge."

The conjoined trabeculæ which together form the cartilaginous basis cranii are continued forwards from the basioccipital as a flat band-like tract forming a floor to the interorbital extension of the cranial cavity. A shallow, longitudinal and median groove, in which the upwardly projecting keel of the parasphenoid lies, extends along this area, and evidently marks the line of coalescence of the trabeculæ. The cartilage forming the bottom of the groove is more transparent than the thicker lateral edges, and hence the primitive distinctness of the trabeculæ is well indicated.

Between the nasal capsules the basis cranii widens out into a quadrangular area formed largely by the subnasal lamina. The postero-lateral angles of this area are occupied by the prefrontals, and the antero-lateral angles by the two bones to be presently described.

The prefrontals (*pr.f*) are separated from the orbito-

sphenoids by a wide tract of cartilage. Internally they bound the foramen for the orbito-nasal nerve, and inferiorly each has a deeply grooved articular surface for the palatine bone. The prefrontals are overlaid by small but distinct ganoid plates (Fig. 1), which appear as the outer and anterior corners of the frontal bones.

The lamina perpendicularis is quite unossified, hence there is no true ethmoid as there is in Polypterus.

The two ossifications above referred to as forming the antero-lateral angles of the internasal area are peculiar to Amia amongst Ganoids. They lie, one on each side of the base of the prenasal process, and appear to be ossifications in the cartilage of the floors of the nasal capsules; inferiorly they rest on the upper surfaces of the vomers.

There can, I think, be but little doubt that these ossicles (*sep.mx*) are homologous with the paired endosteal ossifications, which are to be found at the distal end of the great prenasal rostrum in the Pike. In fact, if the prenasal region in Amia were produced anteriorly into a rostrum comparable to that of the Pike, these bones would exactly resemble in position and relations their homologues in the latter fish. These ossicles would also appear to be homologous with the septo-maxillary bone described by Mr Parker as existing in the floor of the nasal capsules in the Frog; and also with similar bones in the Ophidia. A section carried through these bones and the adjacent cartilage in Amia would resemble in all essentials the various sections given in Mr Parker's paper[1] on the development of the Frog's skull (Pl. x.).

Palato-pterygoid apparatus and suspensorium. (Fig. 6.)

The palato-pterygoid apparatus is constructed on the normal Teleostean type as regards the number and mutual relations of its component bones. It consists of a thin axial core of cartilage which posteriorly becomes continuous with a projecting spur of the quadrate, and anteriorly, in the prefrontal region, swells out into a thickened mass of cartilage and bone overlying the exosteal portion of the palatine. In connection with this

[1] *Phil. Trans.*, 1871.

axial core, palatine, pterygoid and mesopterygoid elements are developed.

The palatine (*pa*) is well developed, greatly exceeding in size its homologue in Polypterus; it is composed of two distinct elements, an exosteal lamina which forms the inferior part and lateral margin of the bone, and an endosteal portion by which the anterior part of the arcade is connected with the prefrontal, and apparently formed by the ossification of a mass of cartilage similar to that which in the Salmon performs a like function. The exosteal element is prolonged forwards in front of the prefrontal bone so as to be ultimately connected with the premaxilla, vomer, and septo-maxillary. It carries two kinds of teeth—long curved teeth arranged along its lateral margin, and resembling those in the premaxillæ, with which they form a continuous series; and a group of short obtusely conical teeth situated internally to those last mentioned, and continuing the series of vomerine teeth.

The endosteal portion of the palatine is triangular in shape, and cancellous in structure. Its superior surface, together with the adjacent cartilage, form an antero-posterior groove for the articulation of the arcade with the prefrontal. The prolongation of the palatine in front of its prefrontal articulation is a well-marked Teleostean modification possessed, so far as is known, by no other living Ganoid.

Behind the palatine the axial cartilage is invested on its inner side by a mesopterygoid (*m.pg*). This bone affords a floor to the orbit. In shape it is triangular, with the apex directed forwards, and overlapping the inner surface of the palatine; its posterior edge is adherent to the inner surface of the metapterygoid, while its superior margin almost touches the outer edge of the parasphenoid. The inner surface of the mesopterygoid is garnished with a number of very small teeth. The pterygoid is an elongated slender bone, which, by means of a small outer and a larger inner plate, clamps the lower edge of the cartilage. Anteriorly this bone unites suturally with the palatine; behind it is applied to the inner side of the distal end of the quadrate, while the outer plate overlaps the inferior two-thirds of the metapterygoid. On the palatine end of the bone there are a few teeth. The tripartite metapterygoid (*mt.pg*) is of

unusual size, and in shape is not unlike that of the Salmon, but instead of lying, as in that fish, immediately over the quadrate, it is situated almost entirely in front of that bone. The small process, which is just indicated in the Salmon, becomes in Amia a conspicuous cartilage-tipped process, rising from the middle of the curved upper surface of the bone, and reaching almost to the level of the cranial end of the hyomandibular. This process seems to represent either the orbital process of the mandibular arch, or the summit of the arch. The pointed anterior end of the bone almost touches the descending process of the alisphenoid; the straight hinder edge is applied to the inner side of the quadrate, reaching nearly to the articular end of that bone[1]. The quadrate has the usual triangular shape. The hinder margin is grooved for the symplectic, and the apex carries a rounded condylar facet for articulation with the mandible. The front edge of the bone is produced into a forwardly projecting process, with which the axial cartilage of the palatopterygoid arcade becomes continuous.

The hyomandibular (*h.m*) does not articulate with any of the otic bones, but is applied to a groove in the cartilage of the otic region immediately over the prootic and opisthotic bones. The posterior border has a strong knob for the operculum, and its middle is obliquely perforated by a foramen for the passage of the posterior division of the facial nerve. The wide synchondrosis which unites the hyomandibular and symplectic bones has a well-marked backwardly projecting "knee," and is grooved by an articular surface for the cartilaginous interhyal. The symplectic (*sym*) is rather large, and is firmly attached, but not anchylosed, to the quadrate. Its cup-shaped distal end furnishes an articular surface for one of the ossicles in the adjacent end of the mandible.

The long axis of the hyomandibular is directed backwards and downwards, and is almost at right angles to the axis of the symplectic, which is directed forwards. It is possible that this angulation of the proximal half of the second postoral arch may account for the forward position of the metapterygoid.

[1] The tripartite shape of the metapterygoid suggests that possibly its three divisions may correspond to the 'ascending process', 'pedicle', and 'otic process' of the Amphibian suspensorium, but the condition of my specimen was such that I could not ascertain the relations of these processes to the branches of the fifth nerve.

The Mandible. (Fig. 7.)

The mandible is an unusually complex structure, as each ramus consists of not fewer than fourteen distinct elements. Meckel's cartilage persists as a thin axial band of cartilage. Its distal end is ossified, and forms a small cylindrical mento-meckelian ossicle (*mt.mk*), which lies in a groove on the inner side of the symphysial end of the dentary (*d*). The proximal end of the cartilage is the seat of at least four distinct ossific centres. Of these, three are arranged in a linear series proceeding from the angular extremity of the mandible. These are referred to in the annexed plates as *a*, *b*, and *c*. Of these the ossicles *a* and *b* form the anterior and posterior boundaries of the articular cup for the quadrate, and are separated from each other by that portion of Meckel's cartilage which forms the bottom of the cup. The bone marked '*c*' is much smaller than the other two. That part of Meckel's cartilage adjacent to the articular cup is produced vertically upwards and forwards into a well-marked "coronoid process" (*cr*). The base of this process is the seat of an ossification (*d*) which forms the outer side of the articular cup, and fits into the cup-shaped distal end of the preoperculum. Thus these three bones, *a*, *b*, and *c*, contribute to the formation of the concave articular surface for the quadrate.

Hitherto it has been currently stated in anatomical text-books that the mento-meckelian bone at the distal end, and the articular bone at the proximal end of Meckel's cartilage, were the only elements of the mandible really formed by ossification of the cartilage itself; yet in Amia there can, I think, be but little doubt that at least four, and probably five, ossific centres are developed in the axial cartilage.

Whether one of the centres *a*, *b*, *c*, and *d* represent the os articulare of the Teleostean mandible, or whether the latter bone is really a compound bone resulting from the coalescence of the persistently distinct elements of Amia, is not very evident; but I am inclined to think that the os articulare is not so simple a bone as it has hitherto been supposed to be.

As the Meckelian cartilage is the distal, or ventral half of the first postoral visceral arch, though it may not be possible to point out the special homologies of the mento-meckelian,

and the ossicles *a*, *b*, *c*, and *d*, with the ossifications found in the ventral halves of the remaining postoral arches, yet I think that we may roughly correlate those ossicles with the interhyal, epihyal, ceratohyal and hypohyal of the hyoidean series.

It may also be that the cartilaginous "coronoid process" is another instance of the tendency manifested by the first postoral arch to develope forward connective outgrowths, of which the orbitar process and the palato-pterygoid arcade are conspicuous examples in the proximal half of this arch.

In addition to the mandibular elements above referred to there are, in addition, several membrane bones. The ossification '*a*' has a small ganoid plate (*d.a*) attached to it, which appears at the extreme tip of angle of the jaw. Just behind this there is a large angular element (*Ag*), and above this splint and applied to the outer surface of the coronoid cartilage there is a supra-angular piece (*s.ag*). The dentary (*d*), shaped like a half cylinder, completes the series of splints seen on the outer side of the ramus. The inner side of the Meckelian cartilage is invested by a large triangular splenial[1] piece, and, as in Polypterus and Ceratodus among Ganoids, and in Siren and larval Salamanders among Amphibia, carries a number of small teeth. Amia also further resembles the young Polypterus, in that the splenial does not extend continuously to the symphysis, but the interval is occupied by a series of five small thin teeth-bearing plates. The splenial teeth, and those carried by the last mentioned ossicles form a continuous series parallel to the much larger, curved teeth carried by the dentary. There is no coronary splint.

Hyoidean and branchial arches.

These structures scarcely, if at all, differ from those of the ordinary Teleostean. The inter-hyal is a small square piece of cartilage attached to a groove in the cartilaginous interspace between the hyomandibular and symplectic. The epihyal has a rounded condyle for articulation with a cup in the adjacent extremity of the interhyal. A ceratohyal and a hypohyal complete the arch.

[1] The splenial element is not shewn in the figures.

There are five branchial arches, and of these the first four are complete, containing pharyngo-branchial, epi-branchial, cerato-branchial and hypo-branchial elements; the fifth arch has the cerato-branchial only represented by bone. The hypo-branchial of the third and fourth arches are bifurcate at the ventral ends. The epihyal of each arch gives off a short, backwardly projecting process which is attached to the pharyngo-branchial of the arch behind it. The basi-branchial elements, four in number, are laterally compressed pieces of bone and cartilage, and only one of them is ossified.

Cranial distribution of the Mucous Canals.

The main lateral canal of each side penetrates the post-temporal, supra-temporal, 'parietal' and nasal bones, and then, instead of joining the transverse ethmoidal branch, as might have been expected, it appears to terminate in three short branches opening at the surface between the nasals, the ethmoid and the præ-orbital bones. Commissural and other branches are given off from each lateral trunk. A transverse canal, traversing the two supra-temporals, connects the lateral canals of opposite sides. A mandibular branch (*s.lc*) leaves each lateral canal in the parietal, and passing downwards through the pre-operculum, as in Teleostei, pierces the angular element, and traverses the dentary to effect a junction with its fellow of the opposite side. Finally, a suborbital branch leaves the lateral canal in the vicinity of the dermo-sphenotic and, perforating that bone, passes downwards and forwards through each of the post-orbital bones, through the suborbital, lachrymal and præ-orbital bones, to the transverse part of the dermo-ethmoid where it joins the corresponding division of the other side.

Numerous pores connect all these canals with the surface at irregular intervals.

Summary.

In summarising the results of the foregoing description of the skull of Amia, I would lay stress on the following facts, as having a special bearing on the affinities of Amia to the more highly specialized osseous fishes and to the Amphibia.

I. The possession of a complete chondrocranium, *i.e.* the absence of fenestræ in the cranial roof, as in Lepidosteus, and the Pike (Esox).

II. The existence of a nearly complete series of otic bones, comprising a large prootic with internal plates forming a characteristic "prootic bridge" in the floor of the cranium, opisthotic, epiotic, and sphenotic elements.

III. The presence of two ossific centres, partly exosteal and in part endosteal, forming rudimentary basi sphenoid.

IV. Septo-maxillary ossifications in the subnasal lamina, as in Clarias, Esox, Rana and Ophidia.

V. The interorbital prolongation of the cranial cavity, separating distinct, paired ali- and orbito-sphenoids.

VI. The prolongation of the palatine in front of its prefrontal articulation and the connection of its anterior end with the inwardly curved process of the maxilla.

VII. The possession of a T-shaped dermal ethmoid overlying the premaxillæ, and the close analogy in number and relations between the investing ganoid plates of Amia and those of the Siluroidei, and especially with those of Clarias, as has been previously described.

VIII. A complete series of opercular bones, a preoperculum anchylosed to the hyomandibular and symplectic bones, an operculum, an interoperculum, and a suboperculum.

IX. The presence of a jugal bone attached as in Teleostei to the upper edge of the maxilla.

X. The existence of a mento-meckelian ossicle, as in Spatularia and the Frog, and of several additional centres of ossification in the proximal extremity of Meckel's cartilage.

XI. The presence of five accessory dentigerous splenial elements in addition to the normal mandibular splints, as in the young Polypterus and Ceratodus among Ganoids, and in Siren and larval Salamanders among Amphibia.

In combining in its cranial structure the anatomical facts expressed in paragraphs I—IX inclusive, Amia differs from all other living Ganoidei, and exhibits distinct and decided affinities to such generalized types of physostomous Teleostei as the Siluroidei, Cyprinoidei, &c. On the other hand, in common with all other Ganoids, Amia possesses several points of

resemblance with larval and adult forms of Amphibia, especially as regards the structures to which attention has been directed in paragraphs IV, X, and XI. Moreover, in the angulation of the mandibular arch caused by the forward growth of its metapterygoid element, we have a repetition of an arrangement characteristic of the adult Frog, and of certain Selachians (Notidanus). But, notwithstanding these evidences of widespread affinity, it is evident that if, in addition to the abovementioned facts, we accredit Amia with the possession of cycloid scales, non-lobate fins, a nearly homocereal tail, and note the absence of spiracles, the Teleostean affinities predominate; and it may be asked whether, despite certain peculiarities in the structure of its generative organs and bulbus arteriosus, the gap between the ganoid Amia and the physostomous Teleostei is not less than need be expressed by ordinal distinction.

It may be that just as Polypterus and its near ally of the same family are the sole surviving examples of the otherwise long extinct order of Crossopterygian Ganoids, so the Amiidæ are the sole survivors of those widely generalized Ganoidei, out of which more specialized Teleostei were directly evolved.

EXPLANATION OF PLATE.

For the drawings from which the accompanying plates were taken, I am indebted to Mr J. W. Clark, whose kindness I gratefully acknowledge. Figures VI. and VII. were drawn for me by Mr C. H. Haddon, of Christ's College. The figures are all of life-size, and the lettering is uniform throughout.

al. s.	alisphenoid.
ag.	angular.
bo.	basioccipital.
b. s.	basisphenoid.
cr.	coronary cartilage.
ct.	cerato-hyal.
d. ptf.	dermo-postfrontal.
d. so.	dermo-supraoccipital.
d.	dentary.
da.	dermal part of 'a.'
eth.	ethmoid.
ep. o.	epiotic.
eph.	epihyal.
ex. o.	exoccipital.
fr.	frontal.
h. m.	hyomandibular.
i. op.	interoperculum.
jg.	jugal.
l.	lachrymal.
Mk.	Meckel's cartilage.
mt. mk.	mento-meckelian.
mx.	maxilla.
m. pg.	mesopterygoid.
mt. pg.	metapterygoid.
a., *b.*, *c.*, *d.*	ossifications in Meckel's cartilage
n.	nasal.
nl. a'., *nl. a''.*	neural arches attached to basioccipital.
os.	orbitosphenoid.
op. o.	opisthotic.
op.	operculum.
pa.	parietal.
pr. o.	prootic.
pt. f.	postfrontal (sphenotic).
pr. f.	prefrontal.
p. op.	preoperculum.
pt. orb.	postorbital.
p. mx.	premaxilla.
p.n.	prenasal process.
pa. s.	parasphenoid.
p. tp.	post-temporal.
pr. orb.	preorbital.
pg.	pterygoid.
q.	quadrate.
s. orb.	suborbital.
sp. mx.	septo-maxillary bone.
s. op.	suboperculum.
s. tp.	supra-temporal.
s. ag.	supraangular.
sym.	symplectic.
vo.	vomer.
II.	Foramen for optic nerve.
V'.	" for the first division of the fifth nerve.
V''., V'''.	" " for the second and third divisions of the fifth nerve.
VII.	" for the facial nerve.

Fig. I. Upper surface of the cranium of Amia with the ganoid plates *in situ*.

Fig. II. Lateral view of the same.

Fig. III. Skull of Amia seen from below. The parasphenoid and vomer are seen, and in addition, the inner side of the palatopterygoid arch; this arch has been removed on the right side.

Fig. IV. Lateral view of the cranium, with the ganoid plates and palatopterygoid apparatus removed.

Fig. V. View of interior of cranial cavity; the ganoid plates and part of the cartilaginous cranial roof have been removed so as to show the basisphenoid and the "prootic bridge."

Fig. VI. Palatopterygoid arcade, with hyomandibular, metapterygoid, quadrate and preoperculum attached.

Fig. VII. Inner side of the mandible: the splenial has been removed in order to show Meckel's cartilage and its accessory ossifications.

From the *Journ. of Anat. and Phys.*, Vol. XI.]

ON THE VASOMOTOR NERVES OF STRIATED MUSCLES. *By* W. H. GASKELL, M.A.[1]

IN a former paper[2] I have discussed the variations occurring in the flow of blood through a muscle of the dog under various conditions. Owing to the nature of the experiment, viz. measuring the flow of blood from the muscle-vein when the muscle was at rest and in action, it was only possible to suggest hypotheses to explain the various phenomena that occurred. I have, however, been able to supplement these experiments with microscopic observations of the vessels in a frog's muscle, and so to clear up many points which were doubtful, and at the same time to show more satisfactorily what is the relation between the nerves and vessels of muscles. These experiments and the discussion upon them form the subject of the present paper.

Since the muscle which I have chiefly used for this purpose, viz. the mylohyoid muscle of the frog, has never before, so far as I know, been made use of for the study of the circulation, it will be better to describe shortly my method of preparation and the nature of the circulation that can be observed in this muscle. I chose a simple muscle like this one rather than the tongue, because the circulation in the tongue cannot be considered as merely a blood stream through muscular tissues, for in addition there are present important glandular elements supplied by the same vessels, and also instead of a single nerve there are various nerves of very different characters, so that the whole question of the nature of the nerve-supply to the vascular tissues is here a much more complicated one, than in the case of the muscles of the dog, which I had used in my former experiments; the tongue therefore is not so suitable for the purpose of supplementing those experiments, as the mylohyoid muscle. Besides this, the circulation in the tongue has already been investigated by different observers without much success.

[1] An abstract of this paper was read before the Royal Society, Dec. 1876, see *Proc. R. Soc.*, Vol. xxv.

[2] *Ludwig's Arbeiten*, 1876, and *Journal of Anat. and Phys.* Vol. XI. p. 360.

By cutting through the skin covering the two mylohyoid muscles in the middle line and then laterally up to both rami of the lower jaw, being careful not to wound the larger vessels in the skin, the flaps of skin can be turned back without any loss of blood, and the two mylohyoid muscles are exposed to view; it is then as a rule seen, that the circulations in the two muscles are nearly if not quite distinct from each other; the aponeurosis separating them containing hardly any vessels except the two large median veins which collect part of the blood from each muscle, and which, running along their respective edges of the aponeurosis, usually turn off to join the other main vessels of each muscle close to the articulation of the upper and lower jaw; and it is seen under the microscope, that the capillaries either form loops at the edge of each muscle and run into veins in the substance of that muscle itself, or else run into their respective median vein. Frequently there is only one median vein present, and sometimes none; in any case, however, the two circulations are distinct. Again, each muscle is attached below to the skin by means of a fine fascia, and here too the main vessels of the muscles pass over this fascia only near the articulation of the two jaws, so that the whole middle part is free from vessels of any size, except in cases when the median veins pass directly across the centre, instead of turning to join the other main vessels; this I have not found often to occur. In order now to observe the circulation in, let us say, the right mylohyoid, the main vessels of the left muscle are first tied and then that muscle is cut through by an incision parallel to the middle line and at a convenient distance from it; next the thin fascia at the base of the muscles is cut nearly up to the place where the vessels of the right muscle cross it, and then, lifting up the right muscle by means of the cut portion of the left, the few very fine connective-tissue fibres attached to the under surface of the muscle are carefully separated; it is now easy to turn the right muscle over on to a prepared diaphragm of gutta percha, and fix it in its place by means of pins stuck into the cut portion of the left mylohyoid; in this way all bleeding is avoided, and the muscle to be observed need never even have been touched. If the frog is supported in the requisite position, it is possible in this manner to spread out the

muscle under a microscope, so as to give a flat surface for examination and at the same time to leave the circulation through the muscle absolutely uninjured. When this has been done, the muscle and its vessels can be examined with nearly the highest powers of the microscope.

The position of the nerve supplying the mylohyoid muscle is also very favourable for experimentation, since arising from the mandibular branch of the trigeminal nerve, it crosses over the ramus of the mandible a little above the articulation, and does not approach the main vessels of the muscle until it has entered into the muscle itself; in order, therefore, to prepare a length of nerve sufficient to be isolated on the electrodes, it is only necessary to cut through the masseter and temporal muscles and so to isolate the mandibular branch of the trigeminal nerve, and at the same time, by cutting away the other smaller branches of this nerve to the skin and other muscles, one makes certain that the mylohyoid nerve alone is stimulated. Thus, then, the nerve can be placed on the electrodes, without any bleeding having been caused and without any interference either to the muscle or its vessels; in fact, my usual plan is to prepare and isolate the nerve, before even cutting through the skin over the two mylohyoid muscles.

In order to obtain an accurate representation of the changes in the calibre of any one artery from time to time, I proceeded as follows. Fixing my attention absolutely on the two thin outer edges of the artery under observation, and on the lines on the micrometer scale, I noted and stated aloud the size of the artery every other second, a metronome on the table beating seconds; these numbers were written down by an assistant, who also opened and shut the key between the induction coil and the electrodes, and took note of any remarks that I might make. Since the animal was always curarized and the nerve well isolated on electrodes protected by paraffin, so that there was no trace of muscular contraction even on strong stimulation, it was easy to measure the varying size of the vessel very accurately at intervals of two seconds, and as this was sufficiently rapid to give a very accurate representation of the variations that occur, I never attempted any more rapid measurements than these. Again, since the stimulation was applied by an

assistant and my whole attention was fixed on the artery under observation, I have often noted the measurements of the artery without being aware of the moment when the stimulation commenced or ended, thus obviating any errors that might arise from an expectation of a particular result.

The spaces between the lines of the micrometer scale represented, with the object-lens that I always made use of, $\frac{1}{120}$th mm., and with this lens the blood-corpuscles were very plainly visible, and the transverse striations of the muscular fibres could be easily seen. As therefore a variation in the diameter of an artery amounting to as much as $\frac{1}{120}$th mm. was by this means very appreciable, I attempted to obtain measurements of smaller variations by dividing with the eye each space into four parts, and estimating the diameter of the artery under observation at any time, according as the variation in its size seemed to correspond most nearly to any one of these four divisions. Variations amounting to as much as one-half or three-fourths of a space, i.e. to $\frac{1}{240}$th or $\frac{1}{160}$th mm., are so visible as to admit of measurement, without much possibility of a mistake as to their occurrence; those, however, that amount only to one-fourth of a space, i.e. to $\frac{1}{480}$ mm., are often more doubtful; as however no inferences have been drawn unless the variations amount to nearly $\frac{1}{120}$ mm., this doubt as to the smallest measurements is of no importance. From the values so obtained, I have constructed a number of curves, the ordinates of which represent the size of the artery every two seconds and the abscissæ the time in seconds. Examples of these curves are given in the course of the paper.

As the study of the circulation in this muscle suggests two distinct subjects of enquiry, I have thought it best to divide this paper into two parts; the first part being a continuation of my former paper and therefore treating of the phenomena that occur in the vascular system of striated muscles, the second part dealing rather with the larger question of the nature of vaso-dilator action.

PART I. THE VASCULAR PHENOMENA OF STRIATED MUSCLES.

1. *The Normal circulation in the muscle.*

Upon examining the muscle when prepared as above described with a low power of the microscope, the nerve being untouched, it is seen that, owing to the thinness of the muscle, the small amount of connective tissue, the absence of glandular tissues and pigment cells, the vessels present an appearance peculiarly favourable for measurement with a micrometer eyepiece; for the edges are so clear and sharply defined, that it is easy to measure accurately the outer edge of the smaller arteries, there being no chance of confusion here between the edge of the artery and the surrounding connective tissue, as in the case of the vessels of the web. If the muscle has been carefully prepared, the blood-stream is seen to have the usual axial character, the inert layer being well seen on each side of the rapid central red corpuscular stream; there is no stagnation in any part of the muscle, but throughout, in arteries, capillaries and veins, a very distinct rapid normal blood-flow is present; and it is possible here to observe the circulation for a long period without any inflammatory changes being set up, and without the character of the blood-stream altering to any great extent; in fact, I have observed the variations in the size of an artery for five hours consecutively, and yet at the end of the time the circulation in the muscle was very nearly as good as at the beginning. Often the arteries seem at first to be rather full and dilated, even though the nerve is intact; soon, however, the stream recovers the normal axial character, and remains in this condition for a considerable length of time, or else gradually and slowly becomes thinner and thinner, until at last there is nearly complete stagnation through the muscle; in the latter case it is only necessary to loosen the pins to see again a good circulation return to the muscle; in fact, extreme care must be taken to prevent as much as possible all strain on the muscle in pinning it out. As to the fulness sometimes seen directly after the muscle has been pinned out, I attribute that to the

effects of some slight carelessness in the preparation of the muscle.

Upon examination of an artery for any length of time, the nerve being untouched, it is often seen that its diameter is continually altering in size, the variations differing very greatly in different animals both as to extent and frequency. They differ from the so-called rhythmic contractions, which have been observed in the vessels of the web and other places, in that the artery under observation suddenly and rapidly dilates, the dilatation in some cases being considerable, and then more gradually returns to what appears to be its normal calibre, the dilatation being always accompanied by a greater fulness of the vessel, the axial character of the stream disappearing.

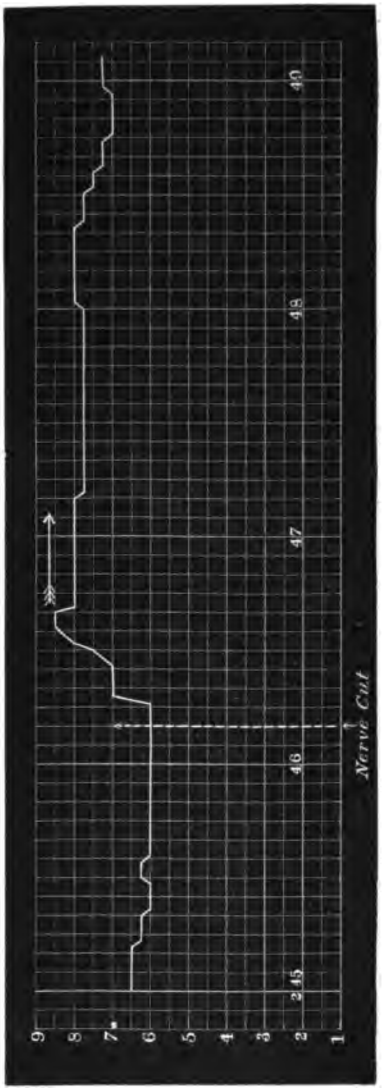
In fact, just as in the arteries of the web the so-called rhythmic contractions present the same appearance as may be produced by slight stimulations at irregular intervals of the sciatic nerve; so here the so-called rhythmic dilatations appear, as we shall see later on, very similar to the effects of a series of slight stimulations of the muscle-nerve; and as in the web and other places, where rhythmic contractions have been observed, they cease as long as the vessel is dilated by section of its nerve, to recommence again as soon as that dilatation begins to subside; so here too the rhythmic dilatations return after the section of the nerve, being in fact often very visible, as Fig. 3 shows.

2. *Effects of section of the nerve.*

Upon removing the skin over the two mylohyoid muscles, it is seen that both muscles are equally pale, and if then one of the muscle-nerves is cut, the corresponding muscle is immediately seen to become redder than the other, the contrast in some cases being exceedingly well marked. If the muscle is first placed under the microscope and the nerve cut while the measurement of the artery under observation continues uninterruptedly, the curve so obtained (see Fig. 1) shows that for a few seconds after section, varying from 5—10 secs., there is no alteration in the size of the vessel either in the direction of dilatation or constriction or in the character of the blood-stream, that then

there ensues a very rapid and considerable dilatation of the artery, which reaches a maximum in about 20—30″ after the section, that this maximum lasts as a rule only a few seconds, and then the artery slowly and gradually returns to its original dimension. During dilatation the blood-stream always loses

Fig. 1.



Curve showing the effect of section of the nerve.

Measurements of the artery taken every other second. Divisions of the abscissa line represent 5 second intervals. The numbers on the ordinate line correspond to the spaces of the micrometer scale, each unit therefore represents an actual size of $\frac{1}{10}$th mm.

its axial character, the lumen of the vessel becoming filled with corpuscles and the rate of flow at the same time more rapid. Throughout the whole muscle the change is very marked, the capillaries are fuller and distended, the venous flow more rapid, the whole circulation more active; as the dilatation subsides the blood-stream again recovers its normal character.

The length of time the dilatation lasts is, as far as I have seen, very variable; it is possible for the circulation to recover its normal character in from two to four minutes after the section, or, on the other hand, for the dilatation to last in a greater or less degree for nearly the same number of hours. In any case the maximum dilatation, which is reached shortly after the section, is not lasting, the more enduring dilatation being always less than that which occurs soon after section; in other words, section of the nerve always causes a considerable temporary dilatation of the arteries of the muscle, accompanied by a more or less permanent slighter dilatation. In accordance with the views already expressed in my former[1] paper, one might ascribe the more temporary dilatation to a stimulation of vaso-dilator fibres by the mere mechanical action of the section; this view is confirmed by the fact, that any mechanical stimulus such as pinching the peripheral end of the nerve, or still more markedly cutting and tearing it with scissors and forceps, is quite sufficient to cause a rapid temporary dilatation of the artery under observation. However, the fact that the dilatation in these cases is temporary, and never so lasting as that which in some cases follows upon section of the nerve, would tend to show that some further explanation is necessary to account for the more enduring slighter dilatation; and this may be found, as I have suggested in my former paper, in the removal of tonicity owing to the section of vaso-constrictor fibres. On the other hand, I do not attach much value to the cases quoted in that paper, as showing a secondary dilatation after nerve section, for I think, as I have mentioned there, that this dilatation was really due to the placing of the nerve on the electrodes[2], since I have repeatedly observed, that this slight stimulation is quite sufficient to cause a notable dilatation in the arteries of the mylohyoid muscle. However, be that as it

[1] *Op. cit.* [2] *Op. cit.* page 374.

may, the fact still remains, that sometimes the artery under observation remains dilated after section of the nerve a much longer time than it would do, if the effect of section was simply that of a mechanical stimulation.

On the other hand, if section of the nerve simply means removal of tonicity, and the gradual diminution of the original dilatation is due to an increase in the elasticity of the vessel-walls, combined with an increased action of peripheral local centres, owing to the greater supply of blood, then the magnitude of the dilatation caused must signify, that the vaso-constrictor fibres in this nerve are in great abundance, or else very strong in action; and upon this hypothesis, if one compares the marked dilatation caused here by the section of the nerve with the slight dilatation caused in the vessels of the web by the section of the sciatic nerve, one would be led to conclude from this fact alone, that stimulation of the peripheral end of the mylohyoid nerve must necessarily cause a much greater constriction of the arteries of the mylohyoid muscle, than any stimulation of the sciatic could cause in the vessels of the web. However, as is shown later on, the reverse is most markedly the case. A second section of the nerve settles this question, for, however carefully and cleanly the nerve may be cut, there always occurs a marked dilatation presenting the same characters and the same maximum as after the first section, with the single exception, that the normal calibre is sooner reached, the dilatation does not last so long.

It is clear then, that one must consider, in the case of this nerve at all events, that a section of the nerve acts like any other mechanical stimulus, as a strong stimulation to vaso-dilator fibres contained in the nerve, and that possibly in addition the dilatation is made more lasting by the removal of the action of vaso-constrictor fibres at the same time. To this question we shall however again return.

3. *Effects of stimulation of the peripheral end of the nerve.*

In my former paper I have drawn attention especially to the six characteristic variations that occur in the curve representing the outflow of blood from the muscle-vein, before, during, and

after the tetanus of the muscle, and have suggested that the outspurt of blood at the onset of the tetanus and the very brief diminution of flow which occurs at the end of the tetanus, are due to the change of form in the muscle from the relaxed to the contracted state and vice versâ; that the absolute or nearly absolute cessation of flow occurring after the first outspurt is over, is due to the pressure of the contracted muscle on some part of the muscular vascular tract, in conjunction with constriction of the arteries, owing to the stimulation of vaso-constrictor fibres contained in the muscle-nerve, this constriction, as Hafiz[1] noticed, being of but short duration; and that the great increase of flow occurring after a short or during the latter part of a long tetanus, is due to the stimulation of vaso-dilator fibres contained in the nerve.

How far these suggestions are true may be decided by observing with a low power the vessels of the mylohyoid muscle in a slightly curarized frog, and then stimulating the nerve so as to cause a decided tetanus of the muscle. It is then seen, that at the onset of the tetanus there is a momentary forward propulsion of the blood in the larger veins, followed by a complete stoppage of the blood-flow in them, or even by a retrograde flow, while in the arteries, with the exception of an absolutely momentary pause, there is from the very commencement of the tetanus a steady rapid flow; instead of any sign of constriction the arteries steadily dilate, the flow in them is fuller, more rapid, the capillaries become full and distended, and at last, even during the tetanus of the muscle, the flow in the veins after a few spasmodic attempts to move onwards recommences, steadily gaining in force and volume, until on the cessation of the tetanus, there is seen through the whole muscle a stream in arteries, capillaries and veins, much fuller and more rapid than before the commencement of the tetanus; at the end of the tetanus, there is a very momentary stop both in the arterial and venous flow. It is clear then that here the same variations in the rate-curve, as were noticed in the case of the dog, would be obtained, if it were possible to measure the outflow of blood from the vein of the mylohyoid muscle of the frog; and moreover that the explanations given in my former

[1] *Ludwig's Arbeiten*, 1870.

paper[1] to account for the six-fold variation of rate observed, agree with what is here observed except in two particulars. In the first place, the change in the form of the muscle, owing to the tetanus, compresses the larger vein-trunks essentially, and therefore the absolute or nearly absolute stoppage of the blood-flow, which occurs at the beginning of the tetanus, is due to compression of this part of the muscular vascular tract; a fact which had been conjectured before, though not proved. In the second place, it is seen that the compression of the vein-trunks is alone sufficient to explain the cessation of flow observed, and that the hypothesis of a previous constriction of the arteries lasting only for a short time, in consequence of a rapid exhaustion of constrictor fibres stimulated at the same time as dilator fibres, is not only unnecessary, but is, in fact, not true; for there is no sign of any constriction of the arteries at the commencement of the tetanus, but on the contrary a steady dilatation.

Applying now the results of this experiment to the facts and curves given in my former paper, it is seen that the difference noted between the effects of a short and long tetanus simply means, that the compression of the larger veins due to the contraction of the muscular fibres is sufficient for a certain length of time to counteract the increase of flow, that would otherwise take place in consequence of the dilatation of the arteries; and that after that time, the pressure of the blood inside the veins, which has been steadily increasing, is able more and more to overcome the external pressure exerted by the contracted muscular fibres, and therefore the flow of blood from the veins steadily increases even during the tetanus itself. Again, in those cases represented by Fig. 6 of that paper[2], where the increase of flow is manifested from the very beginning of the tetanus, and where the outspurt at the onset is wanting, the explanation is easily found in the supposition, that the tetanus here was so weak as not to cause a sufficient compression of the larger veins, and that therefore the effect of the dilatation of the arteries was made manifest from the commencement. In fact, on looking over the curves representing this effect, I see that I have always noted that the tetanus was weak when a

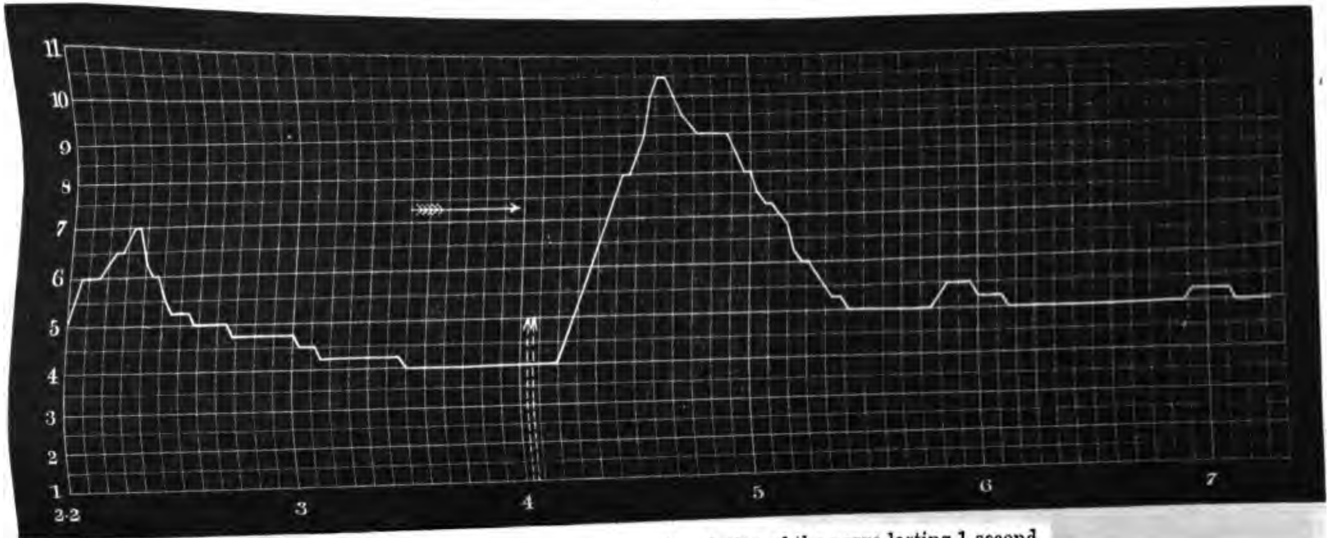
[1] *Op. cit.* [2] *Op. cit.* page 381.

curve like Fig. 6 was produced. Further, Fig. 3 in the same paper, which shows the effect of the tetanus of 0·4 seconds' duration, can only be explained on this hypothesis; for it is impossible to conceive, both that unstriped muscular fibres contract slowly, a fact which is well known, and yet that the contraction of the arteries in the extensor muscles, due to the stimulation of vaso-constrictor fibres contained in the crural nerve, is over and gone in the space of 0·4 seconds, although at the same time with the same length of stimulation the action of the vaso-dilator fibres does not reach its maximum until between 10 and 15 seconds after the stimulation is over.

The nature of the changes taking place in the vessels of the muscle, in consequence of the stimulation of its nerve, can be more efficiently studied by using larger doses of curare; for it is possible then to stimulate the nerve with even strong induction currents, without obtaining the slightest trace of muscular contraction, while at the same time the vasomotor nerves are unaffected by the curare, and so one is able to use a high power of the microscope and measure to the smallest variation the changes in calibre of the artery under observation. By the method described above, I have obtained a great number of curves showing the changes occurring under different conditions. Of these I give the following examples, in order to show the nature of the results obtained. (See Figs. 2, 3, 4 and 5.)

By studying these and similar tracings, one finds that whether the stimulation is strong or weak, long or short, there is always a certain time after its commencement during which there is no effect produced on the artery under observation; during this 'latent period,' which is of variable length, but lasts as a rule 5 or 6 seconds, there is no sign of constriction, except that, if the artery happens to be constricting when the stimulation is applied, it will continue that constriction during this period, just as if it be dilating, it will continue that dilatation, and if it be at rest, it will remain at rest. Following upon this latent period, a very rapid marked dilatation of the artery takes place, the vessel becoming crowded with corpuscles and the stream in it very full and rapid; the dilatation in some cases being so great that the diameter of the artery increases to nearly three times the size it possessed before the commencement of the stimulation

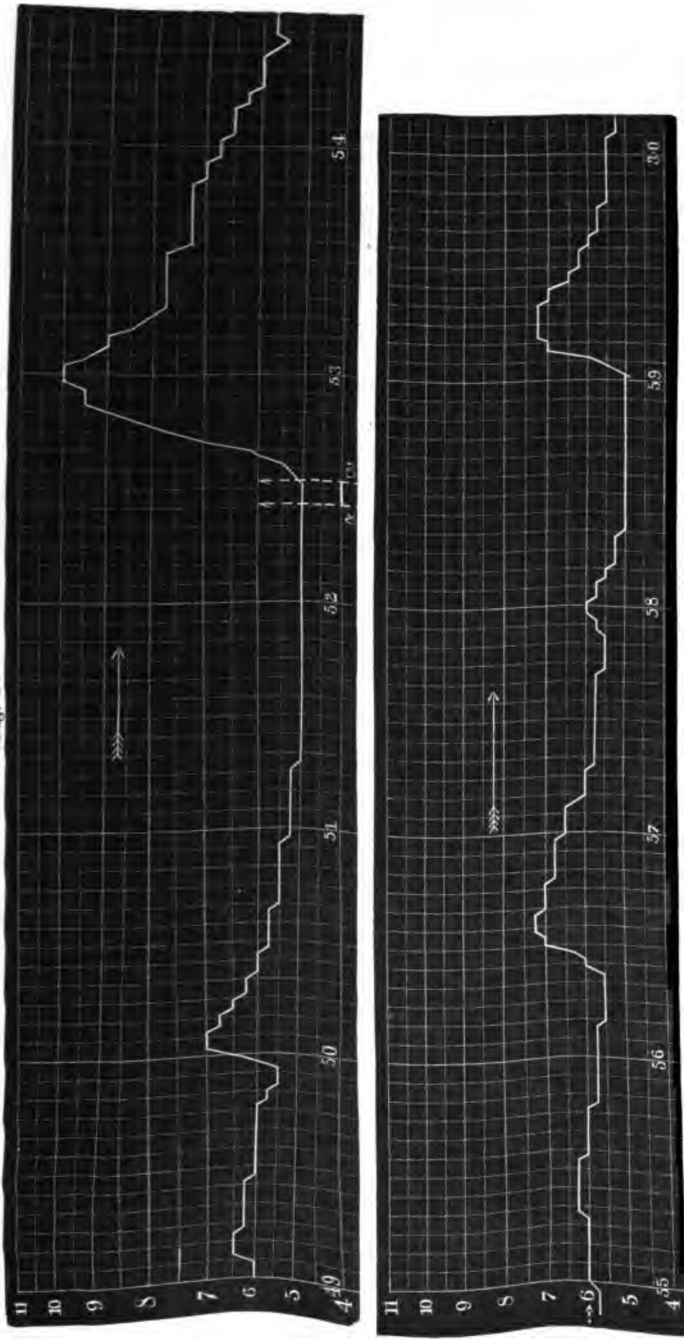
Fig. 2.



Curve showing the effect of a strong stimulation of the nerve lasting 1 second.

Measurements of the artery taken every other second. Space between the two dotted lines represents the time of stimulation. Ordinate and abscissa divided as in Fig. 1. At the commencement of the tracing one of a series of "rhythmical dilatations" is shown.

Fig. 3.

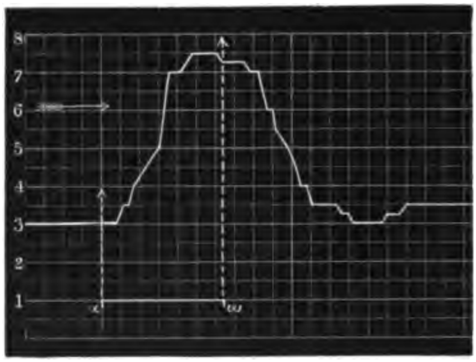


Curve showing the effect of a strong stimulation of the nerve lasting 6 seconds. The lower curve is the continuation of the upper one.

Abscissa and ordinate lines divided as in Fig. 1. Measurements of artery taken every other second. Stimulation of nerve commenced at *a* and ended at *ω*.

The curve also shows the "rhythmical dilatations" both before and after the stimulation of the nerve.

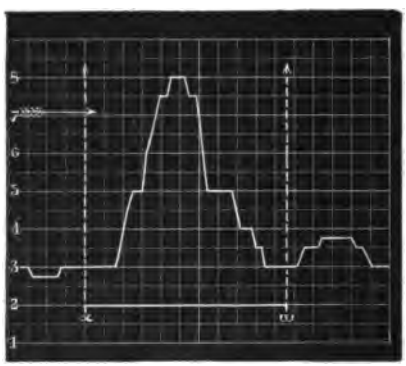
Fig. 4.



Curve showing the effect of a weak stimulation of the nerve lasting 32 seconds.

Abscissa and ordinate lines divided as in Fig. 1. Artery measured every other second. The stimulation of the nerve commenced at *α* and ended at *ω*.

Fig. 5.



Curve showing the effect of a moderately strong stimulation of the nerve lasting 54 seconds.

Abscissa and ordinate lines divided as in Fig. 1. Measurements of artery every other second. Stimulation of nerve commenced at *α* and ended at *ω*.

(see Figs. 2 and 5). The dilatation attains its maximum as a rule about 20—30 seconds after the commencement of the stimulation, and then after remaining at this point for a few seconds gradually subsides, the character of the stream again becoming normal. It is clear then, from the many exactly similar observations which I have made, that stimulation of the mylohyoid nerve causes a marked dilatation of the arteries of the muscle without any previous constriction. It is possible perchance to imagine, that the curare has made the constrictor fibres inactive and yet left the dilatator nerves intact; that this supposition will not hold good, is seen by stimulating the sciatic nerve at the same time and with the same strength of stimulation as the mylohyoid nerve, and then by observing the web and muscle at the same time, it is seen, that a stimulus which causes a dilatation in the smaller arteries of the muscle to nearly three times their original calibre, causes a constriction of the arteries in the web, so as absolutely to close their lumen, and to prevent any corpuscles from passing along. The marked contrast between the colourless absolute stagnation in the web, and the rapid full circulation in the muscle under the same conditions, is exceedingly striking. Moreover, as in the muscle it is seen that some time elapses—about 30 seconds—before the maximum of dilatation is reached, so in the web much the same time elapses before the maximum of constriction is attained. Therefore by stimulating the sciatic and the mylohyoid nerves at the same time with a strong stimulation lasting only a fraction of a second, it is easy to see, after the end of the stimulation, the progressive increase of the constriction on the one hand, and of the dilatation on the other; that is to say, that as the dilatation caused by stimulation of the muscle-nerve is not confined to the time of stimulation, so the constriction in the arteries of the web is not confined to the small time during which the electrical current is passing. Now it is impossible to consider, that the action of the vaso-constrictor nerves in the muscle can be very different from those in the web; at all events one cannot conceive, that a constriction can possibly take place in the arteries of the mylohyoid muscle, which should last so short a time as not to be observed, either during the stimulation itself in the case of a longer stimulation, or after the stimulation is over in the case of a stimulation lasting

less than one second. Besides, there is no sign of constriction when chloral alone has been given without curare. Therefore it seems to me that the supposition of Hafiz, that the vaso-constrictor nerves of muscle are very easily exhausted, is absolutely insufficient to account for the fact, that there is no sign of constriction after a stimulation lasting not longer than a fraction of a second.

Either then there are here in the mylohyoid nerve only vaso-dilator fibres, or else upon simultaneous stimulation of the two kinds of fibres the vaso-dilator always get the mastery, the exact reverse of what v. Frey[1] has observed in the case of the simultaneous stimulation of the sympathetic and chorda tympani, although agreeing according to present theories somewhat with Baxt's[2] observations on the effects on the heart of simultaneous stimulation of the accelerans and vagus nerves. There are only two cases in which I have succeeded in obtaining any satisfactory sign of constriction in the mylohyoid vessels in consequence of nerve stimulation; the first instance is the constriction mentioned below, which occurs after a long strong stimulation, the second that occurring upon reflex action, which will be discussed later on.

As I have already said, the maximum of the dilatation caused by stimulation of the nerve takes place about 30 seconds after the beginning of the stimulation, and this is true whether the stimulation lasts for a short time or a longer one, so that it is possible in the case of a short stimulation lasting only 5 seconds or less, that the whole dilatation should occur after the stimulation is over. On the other hand, as Fig. 5 shows, in the case of a longer stimulation the whole dilatation may occur during the time the current is passing, so that when the stimulation is ended, the artery under observation is found to have regained its normal character; that is to say, the extent of the dilatation caused by stimulation of the nerve depends rather on the strength of that stimulation, than on the duration of it. It is not possible however with the same strength of stimulation, however strong that may be, to make the dilatation last for any length of time; although I have succeeded in keeping the artery under observation in a state of maximum dilatation for

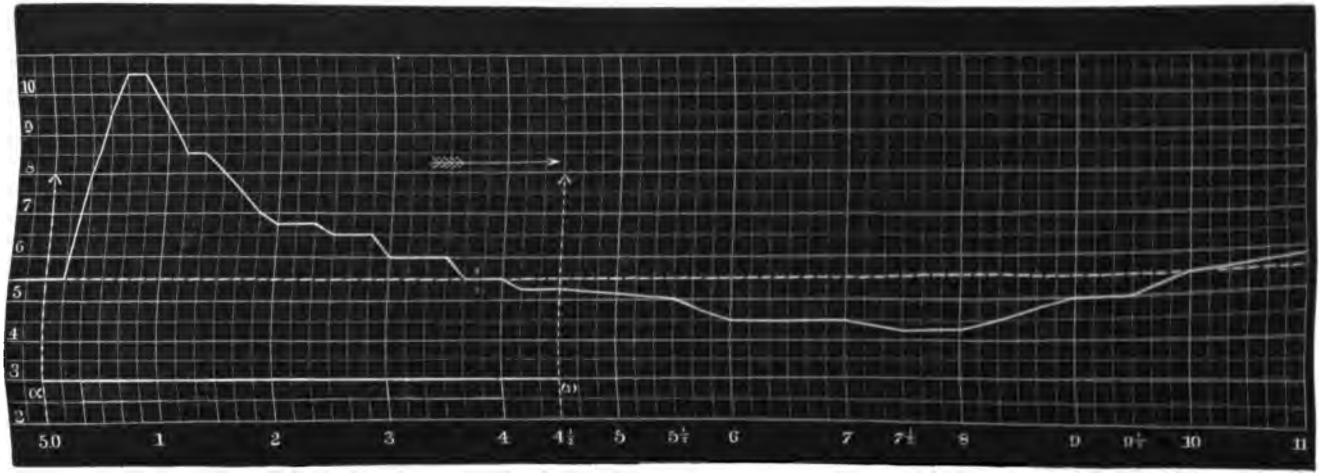
[1] *Ludwig's Arbeiten*, 1876. [2] *Ibid.* 1875.

as long a time as 5 minutes, by commencing with a weak stimulation, and continually and very gradually increasing the strength, whenever the dilatation showed signs of diminishing; on the other hand, in the case of a strong stimulation of unvarying strength lasting 4½ minutes, it is seen (Fig. 6) that before the end of the stimulation the calibre had returned to the normal size, the dilatation caused in fact being very much the same in extent as would have been caused by a stimulation of the same strength, lasting a much shorter time. The curve however shows another circumstance of great interest, which occurs as a rule after any stimulation that has been long enough and strong enough, viz. that upon the cessation of the stimulation, the artery not only does not dilate but steadily continues to diminish in calibre, until at last, some time after the end of the stimulation, it has reached a size considerably less than that which it possessed before the beginning of the stimulation; after remaining at this minimum for a short time, the vessel again gradually returns to its normal size or even slightly above it. We see then, that whereas in the case of the web a long and strong stimulation of the sciatic nerve causes constriction of its arteries, followed after the stimulation by dilatation, so here the same cause produces dilatation of the arteries of the muscle, followed after stimulation by constriction. One would rather then imagine that, as a consequence of any stimulation of such a nerve as the sciatic, constriction of the arteries of the skin is accompanied by dilatation of those of the muscles, and dilatation of the skin-vessels by constriction of those of the muscles. How far in this way a compensation takes place between the two vascular areas of the skin and muscle in any part of the body, will depend upon the degree in which the two areas are supplied by the nerve, whose action is being investigated.

A similar occurrence to this secondary constriction has been observed by Hafiz[1]. He noticed that the dilatation of the arteries in muscles caused by a strong stimulation of the upper portion of the spinal cord was followed by a very marked constriction of them; and in his paper this seems to me to be the only satisfactory constriction of the muscle arteries that he observed; for, although he says that he has sometimes seen a more or less

[1] *Op. cit.*

Fig. 6.



Curve showing the effect of a strong stimulation of the nerve lasting 4½ minutes.

The ordinate line divided as in Fig. 1. Each of the divisions of the abscissa line represents 10 seconds. Stimulation of the nerve commenced at *α* and ended at *ω*. Measurements of the artery taken every 10 seconds until the end of the stimulation, then every minute or half minute. For some 5 minutes before the stimulation commenced the diameter of the artery had measured 5 or 5·5, the fluctuations in its size being very slight.

temporary constriction of the muscular branches of the ulnar artery, previous to the marked dilatation caused by stimulation of the spinal cord—and upon this he founds his assumption that the vaso-constrictor nerves of the muscle arteries are exceedingly easily exhausted—yet he expressly says, that he has never seen the slightest trace of this constriction in curarized animals, although at the same time there is marked constriction in other arteries, and also, that as a rule stimulation of the upper portion of the spinal cord causes no constriction of the muscle arteries, but, on the contrary, causes a great increase in the amount of blood flowing from the cut surface of the muscle. Hence it seems to me plain, that in those cases where he noticed constriction of the muscle arteries upon stimulation of the spinal cord, there must have been at the same time a contraction of the muscle; and that therefore, whichever of his different methods was the one by which he noticed the fact of constriction, the mere presence of a simultaneous contraction of the muscle must have made it very difficult, to say the least, for him to have been sure that what he observed was really due to the stimulation of vaso-constrictor fibres of the muscle arteries. In the case of a curarized frog, stimulation of the medulla causes a decided dilatation of the arteries of the mylohyoid muscle without any previous constriction, although at the same time the arteries in the web are strongly contracted; and if the mylohyoid nerve be first cut, then stimulation of the medulla remains without any marked effect, there being certainly no constriction although possibly a very slight dilatation produced.

4. *Effects of reflex stimulation.*

As I have mentioned in my former paper, it does not follow that, because contraction of a muscle is accompanied by dilatation of its arteries when the muscle-nerve is artificially stimulated, the same thing should occur in the case of a contraction of the muscle caused by voluntary or reflex stimulation. As it is not possible in the frog to examine the muscle and note the effects of voluntary muscular contraction, one is compelled to confine oneself to the question, what is the effect of reflex action on the arteries of the mylohyoid muscle.

As is well known, stimulation of a sensory nerve causes a great increase in the blood-pressure of the larger arteries, owing to a reflex constriction of a large number of the smaller arteries in the body. A very indirect proof, that the arteries of muscles do not partake in this previously supposed nearly universal constriction, is given by Hafiz; whether however the muscle-arteries simply remain unaffected, or are partially constricted, or whether they absolutely dilate upon stimulation of a sensory nerve, his method of observation is insufficient to show; he simply proves, that all blood channels are not occluded, when by different means he causes a great rise of pressure in the carotid; he concludes thereupon, that the open paths for the blood stream are situated in the muscles. By my method of observation, I was able to test how far this theory is true for the frog, at the same time being able to control the effect of the stimulation, by observing simultaneously the circulation in the web and the muscle. The nerves, whose central ends I stimulated with varying strengths and durations of stimulations, were the two sciatics, the vagi, and the opposite mylohyoid, and in no case was there any dilatation of the arteries under observation to be seen; either no change at all was produced, or else together with a diminution in the rapidity and fulness of the blood stream a slight very gradual constriction of the artery was observed. At the same time it was seen, that the vessels of the web constricted notably when those in the muscle remained unaltered, and that in the case of stronger stimulation where there was a very slight constriction of the mylohyoid arteries to be observed, those of the web were absolutely occluded and the whole circulation there stopped; this was true, not only when the central ends of the sciatic and vagi nerves were stimulated, but also when the nerve made use of was the opposite mylohyoid; it being no more possible to produce any marked effect on the muscle arteries by the use of this latter nerve, than by the use of any other sensory nerve. It seems then clear, that stimulation of sensory nerves, while causing a marked constriction in the arteries of the web, does not affect the arteries of the muscles to any great extent in either one way or the other, what effect there is being rather in the direction of constriction than

dilatation. That there is a slight constriction caused seems probable from the fact, that although if the muscle-nerve is left intact stimulation of an ordinary sensory nerve never produces a trace of dilatation in the artery under observation, but either no effect or else a slight constriction, yet when the muscle-nerve has first been cut and a sufficient time elapsed for the circulation to have recovered its normal character, then stimulation of an ordinary sensory nerve sometimes causes a slight dilatation of the muscle-arteries, owing clearly to the greater amount of blood sent through the muscle in consequence of the marked constriction occurring in other parts of the body; therefore, even in those cases where the stimulation of a sensory nerve appears to produce no effect on the calibre of the muscle-arteries, this very fact shows, that in reality the vaso-constrictor fibres in the muscle-nerve must have been thrown into action to such an extent as to counteract the dilatation, that would otherwise have taken place in consequence of the increased flow of blood through these vessels.

From this fact then, that stimulation of a sensory nerve does not cause dilatation of the vessels of the muscle, it would seem to follow that a contraction of the muscle caused by reflex action is not accompanied by a stimulation of the vaso-dilator nerves of the muscle. Before however one can speak with certainty on this point, it is necessary to make sure that there is no particular locality, stimulation of which will produce dilatation of the arteries in question. We know already a considerable number of local reflex dilatations of vessels; thus stimulation of the central ends of the saphena and great auricular nerves of the rabbit causes a dilatation of the saphena artery and of the median artery of the ear respectively (Lovén)[1], and in the case of the submaxillary gland, stimulation of the tongue or central end of the lingual nerve causes a reflex dilating action of the chorda tympani (Vulpian). This latter case seems especially to shew, that the same cause which calls the function of an organ into play calls into action the vaso-dilator nerves of that organ; therefore applying this to the case of the mylohyoid muscle, it is clear that the most likely method to obtain a reflex dila-

[1] *Ludwig's Arbeiten*, 1866.

tation of its vessels is to apply a stimulus to those places, where it is easiest to obtain a reflex contraction of the muscle. One must then first find out, where these most favourable spots are; now the two mylohyoid muscles are essentially muscles of respiration and deglutition, and by first removing the cerebrum and optic lobes in a non-curarized frog, so as to obtain the phenomena of reflex contraction in the purest manner, it is easily seen, that a small piece of blotting paper dipped in 5 per cent. solution of acetic acid placed either at the opening of the glottis or œsophagus, immediately causes a strong reflex contraction confined to these muscles and their neighbours, while the same stimulus applied to other parts of the body has no effect as far as these two muscles are concerned. Stimulation here then causes a reflex contraction of these muscles, is this accompanied by a reflex dilatation of the arteries or not? This question must, I think, be answered in the affirmative, for although I have not succeeded in obtaining as clear and constant evidence of dilatation by stimulation here, as by stimulating the muscle-nerve itself, yet I have sometimes seen decided dilatation of the vessel under observation on the application of a stimulus to these parts; and as a rule this much can be said, that after the entrance of the œsophagus or glottis has been stimulated in any way, the artery measured is for some time afterwards slightly larger, and the stream in it certainly fuller and more rapid than before the stimulation. The most satisfactory and most pronounced dilatation that I have seen has been, when the glottis was opened and a blunt instrument such as a seeker passed into the interior of the larynx; in these cases I have seen a distinct dilatation take place not only after the instrument has been withdrawn, but also while it was still held in position within the opening of the glottis. At present therefore I am inclined to think that the arteries of the mylohyoid muscle of the frog behave in accordance with what may prove to be a general law, viz. that stimulation of a sensory nerve causes dilatation of the smaller arteries of that part, with which it is in functional relation, and constriction more or less marked of the remaining smaller arteries of the body. As it is however very advisable to determine whether the

vascular system of the muscles of mammals conforms to the law of reflex action here laid down, before speaking positively on the subject I will defer further discussion, until the completion of certain experiments on curarized mammals, by which I hope to obtain a solution of this question; for I cannot help thinking, that I have laid too much stress upon the single curare experiment mentioned in my former paper[1], and that it is very improbable that curare should destroy the action of vaso-dilator fibres in the case of mammals, while leaving them intact in the case of frogs, especially as Hafiz mentions, that in many of his experiments he made use of curare, and yet does not lead one to conclude, that in these cases the dilatation effects noticed by him in the vessels of muscles upon stimulation of the cord were absent, although he expressly states that the preliminary constriction was always wanting. Further it is not possible even by large doses of curare to paralyse the dilator fibres in the mylohyoid nerve of the frog, for I have purposely given large doses of curare at one time and also many times the usual dose by means of injections repeated at intervals, and yet have always found that the vessels of the muscle dilated in response to stimulation of the nerve.

As Claude Bernard has described a dilatation of the branches of the facial artery upon stimulation of the mylohyoid nerve in the case of the mammal, I have experimented in the same manner upon two other muscles of the frog, viz. the abdominal portion of the pectoralis major and the lateral portion of the rectus abdominis muscles, and have seen in each case dilatation of their arteries upon stimulation of their respective nerves. As however the preparation of these muscles is not so easy as that of the mylohyoid, I have not as yet extended my experiments upon their vasomotor nerves. I have however seen enough to justify the assumption that the phenomena described in this paper are not confined to the mylohyoid muscle. In the case of the tongue I am inclined from the few experiments I have made to agree with v. Frey[2], that stimulation of the glossopharyngeal, rather than the hypoglossal nerve, causes vascular dilatation here, as also Vulpian has proved for the mammal.

[1] *Op. cit.* page 372. [2] *Op. cit.*

To recapitulate, the following conclusions seem to result from the foregoing experiments.

Every muscle-nerve contains vaso-motor nerve fibres, consisting chiefly of dilator fibres, the constrictor fibres being very weak in comparison to the dilator.

Section of the nerve acts like any other mechanical stimulus and causes a stimulation of these dilator fibres.

The presence of constrictor fibres can only be shown indirectly, for when the two kinds of fibres are simultaneously stimulated by placing the whole nerve on the electrodes the constrictor fibres are not able to overpower the dilator and make their presence manifest, and so dilatation alone is caused.

The constrictor fibres are assumed to be present, because the dilatation caused by section lasts sometimes too long a time to be explained by stimulation of dilator fibres alone, and therefore must be explained by the removal of tonicity, and also because slight constriction may occur in consequence of reflex stimulation; both of which facts necessitate the presence of constrictor fibres.

The muscle arteries of the frog follow to a slight extent the general law of constriction upon stimulation of a sensory nerve, dilatation only occurring upon stimulation of that sensory area with which the muscle stands in functional relation, thus suggesting the general law, which is borne out by many other observed instances of dilatation caused by reflex action, that stimulation of a sensory nerve augments the action of the vaso-motor centre as a whole, but inhibits that part of it which regulates the particular area which stands in functional relation with that sensory nerve.

Stimulation of the vaso-motor centre itself in the medulla causes not constriction, but dilatation of the arteries of the mylohyoid muscle of the frog, probably because owing to the course of the fibres the dilator fibres in the mylohyoid nerve are stimulated at the same time.

As in the web and many other places the constriction caused by strong stimulation of constrictor fibres is followed by dilatation, so in the muscle the dilatation caused by strong stimulation of its nerve is followed by constriction.

As rhythmical constrictions of the arteries have been observed in the frog and rabbit, in places where constrictor fibres appear to predominate, so in the muscles of the frog, with the preponderance of dilator fibres, rhythmical dilatations are seen; in both cases the appearances resemble those that would be caused by slight stimulations at irregular intervals of the predominant kind of nerve fibre.

PART II. ON THE NATURE OF VASCULAR DILATATION.

It is clear from what has been stated in the first part of this paper, that, looking to the magnitude of the dilatation and the ease with which it can be produced and observed, one possesses in this muscle a very favourable opportunity of studying the nature of vascular dilatation. Two chief rival theories have been suggested to account for vaso-dilator action: the 'active dilatation' theory and the 'inhibition' theory. In the first, the nerve is supposed to exert its influence directly on the walls of the artery, and by some unknown mechanism to cause the vessel to dilate, producing thereby an increased flow of blood; in the second, which is the generally accepted one, the vaso-dilator nerves, by some inhibitory action, are capable of removing the tonicity of the arteries, and thereupon the pressure of the blood within them causes a distension of their walls; that is to say, the amount of dilatation caused by stimulation of vaso-dilator nerves is on this hypothesis dependent on the blood-pressure in the vessels.

Now it frequently happens, in consequence of slightly overstraining the muscle when pinning it out for observation under the microscope, that the circulation through it gradually becomes worse and worse, until at last the artery under observation appears absolutely empty of blood-corpuscles, although at the same time it is by no means closed; upon now stimulating the muscle-nerve with a strong tetanizing current, the animal having previously been curarized, the empty artery is often seen to dilate steadily, until sometimes it has attained to nearly double the size it possessed previous to the stimulation, before a single

blood-corpuscle makes its appearance; then a rush of corpuscles takes place, the artery is dilated to its full extent, and now over the whole field of view is seen a rapid active circulation. In fact, whenever the circulation is very poor, the arteries being partially constricted and the blood-stream thin and slow, it is only necessary to stimulate the nerve to produce at once a full rapid circulation through the whole muscle. As this observation appeared to point to the possibility of obtaining dilatation of an artery when the blood-pressure was removed, I next proceeded to get rid of the arterial pressure by ligaturing the aorta before stimulating the nerve. In order to show the effect produced, I give as an example the details of one observation. The numbers represent the spaces on the micrometer scale, each of which denotes an actual size of $\frac{1}{120}$th m.m.

Nov. 29, 1876. $2\frac{1}{2}$ drops of $\frac{1}{2}$ per cent. solution of curare injected under skin of male frog at 9.30 a.m.

Right mylohyoid nerve cut at 12 noon, and right mylohyoid muscle, which was seen to be redder than left, prepared. Heart exposed and ligature placed under bulb of aorta, muscle fixed under microscope and nerve put on electrodes protected by paraffin.

At 12.57 p.m. A very fair circulation through muscle, vessels full and slightly dilated. Artery chosen for observation, measured at

1.1 p.m. 4·25.

1.2 p.m. 3. On then stimulating the nerve with moderately strong current for 30 seconds, the artery dilated steadily to 7, with no trace of muscle contraction.

At 1.4 p.m. it had diminished to 4; the bulb of the aorta was now clipped, and

At 1.5 p.m. the diameter had diminished to 3, there being still a slight movement of corpuscles in the right direction.

1.6 p.m. 3. Still a perceptible flow of corpuscles; the nerve was now stimulated for 40″ with the same strength of current as before; there was no muscle contraction; the artery dilated steadily to 5·75, becoming filled with corpuscles, which flowed in from the venous side, and at the very end of the stimulation the slow flow was again reversed, the vessel beginning to contract; this contraction continued so that

At 1.9 p.m. the artery measured 3·25, being very nearly empty, and the few corpuscles in it quite stationary. The nerve was now again stimulated for 45 seconds with a stronger current; there was a slight trace of contraction of the muscle, and a steady dilatation of the artery to 5·25, with a slow flow of corpuscles from the venous side, which continued until very nearly the end of the stimulation, when, with the commencing constriction of the artery, the corpuscles moved again in the right direction; and

At 1.11 p.m. the calibre measured 3·25, with a few stationary corpuscles in the vessel.

It is clear then from this and similar experiments, that dilatation of an artery can take place on nerve-stimulation, even after the arterial pressure has been removed by clipping the aorta, although the amount of dilatation so caused is always less than the same stimulus produces when the circulation is intact; it is possible therefore to attribute the dilatation noticed under these circumstances, on the hypothesis of inhibition, to the pressure which is still present in the whole vascular system in consequence of the whole amount of blood being still contained in the vessels. In order to test more efficiently the possibility of obtaining dilatation of an artery in the absence of all blood-pressure, I next proceeded not only to arrest the circulation through the muscle, but also to remove as far as possible all blood from its vessels. To effect this, I first exposed the heart and cut through the large veins, so that the still beating heart might drive as much blood as possible through the cut ends of the veins; I then cut through the aorta and removed the heart bodily. As however if the muscle is now prepared, it is not easy to be sure of finding an artery convenient for observation, on account of the bloodless condition of the muscle, my usual plan was to prepare the muscle and its nerve, before cutting out the heart, whereby too the advantage was gained, of being able to observe the amount of dilatation caused by a given strength of stimulation, when the full circulation was present, and therefore to compare this with the effect produced in the same place of the same vessel by the same stimulation, when the heart had been removed. The behaviour of the artery under observation after removal of the heart is not always the same. Sometimes a slow steady constriction takes place, which may reach its limit a few minutes after the removal of the heart, the artery then being nearly closed and the few corpuscles remaining stationary in it; during the constriction the corpuscles are slowly propelled onwards; sometimes the slowly progressing constriction is interrupted at irregular intervals by rapid sudden dilatations of the vessel, thus reproducing on a smaller scale what I have called "rhythmical dilatations"; the movement of the corpuscles owing to the constriction is always

reversed during each of these shortly lasting dilatations, and at the end the artery remains constricted and the corpuscles stationary as in the first case; if, as sometimes occurs, probably from some peculiarity in the tension of the muscle, the corpuscles are moving in the reverse direction, i.e. from the veins to the arteries, during the constriction of the vessel, then with each dilatation the motion is changed to the normal direction; sometimes the artery under observation is found to be absolutely constricted directly after the removal of the heart; this I have only noticed in three cases when the heart had been cut out previous to the section of the muscle nerve, and in each case subsequent section of the nerve was without effect, the artery remaining in the same state of absolute constriction. Upon stimulation of the nerve, either when the artery is at the end of its constriction or while it is still constricting, a marked dilatation of the vessel is often seen to take place, the corpuscles moving in a direction the reverse of their motion during constriction; the amount of this dilatation, though sometimes as great as the doubling of the calibre the vessel possessed just before the stimulation, is yet always less than that produced by the same stimulation when the full circulation is present, and is often sufficient only to enlarge the vessel up to that size, which might be considered the normal one previous to the removal of the heart. In one of the three cases above mentioned where the artery remained absolutely closed after section of the nerve, the unstriped muscular fibres could be clearly seen projecting along the edge of the artery and the inner lumen was clearly marked by two lines nearly touching, so that by using a power, with which every space on the micrometer scale corresponded to $\frac{1}{244}$ m.m., the whole vessel measured 5, each muscular wall representing 2 and the inner lumen 1. Upon stimulation of the muscle at the point of entrance of the nerve, the hitherto closed vessel was made to open and finally after two or three stimulations the size of the vessel had reached 7, cf which the inner lumen represented 5 and each muscular wall only 1: at the same time the separate projections of the contracted muscle fibres nearly disappeared, so that the outer edges of the vessel were now almost as smooth as those of the inner lumen. As the vessel dilated two corpuscles

made their appearance coming from the venous side, which when the vessel again began slowly to constrict moved again towards the veins; as long as the observation lasted the artery now remained open, the inner lumen never becoming less than 3.

The foregoing experiment does not enable us to decide between the two rival theories, which have been put forward to explain vaso-dilator action, for, although on the "active dilatation" theory it is easy to see how the stimulation of the peripheral ends of vaso-dilator fibres might occasion a dilatation of an artery, while at the same time the removal of tonicity implied by the section of the nerve was unable to do so; yet it is also quite conceivable, that the section of the nerve, i.e. the removal of the action of some cerebro-spinal vasomotor centre, was unable to produce any effect, because, in consequence of the absence of the blood-supply, the tonic influence of the peripheral local vasomotor centres was so much increased, that this alone was able to hold the vessel in a state of constriction; while, on the other hand, stimulation of the peripheral end of the nerve caused the vessel to dilate, because the action of the peripheral local centres was thereby inhibited. As an alternative to this view, it may be urged, that possibly the mylohyoid nerve contains only a few vaso-constrictor fibres, and that the majority of these fibres take some other course, as for instance directly along the vessels of the muscle themselves, so that section of the nerve in the case cited was unable to produce any effect, because the supposed other constrictor fibres were thrown strongly into action by the stimulation of the cerebro-spinal vaso-motor centre, in consequence of the absence of its blood-supply; while stimulation of the peripheral end of the nerve removing the action of all constrictor fibres allowed the artery to relax; and as a proof of the existence of these fibres, the fact might be urged, that it is ordinarily possible to produce a greater dilatation by stimulation of the nerve, than by section of it, and therefore that the mylohyoid nerve does not contain all the constrictor fibres of the arteries; also the varying length of time that dilatation lasts after section of the nerve would be explained by supposing that sometimes more sometimes less constrictor fibres are contained in the nerve itself. Against this possibility however is the fact, that stimu-

lation of the upper portion of the spinal cord is absolutely without effect when the mylohyoid nerve has first been cut; also, if there were nerves of this character situated upon the vessels themselves, it seems reasonable to suppose, that direct stimulation of an artery, by not only stimulating them, but also stimulating the muscular coat of the vessel itself, ought to produce a marked contraction of the vessel. I therefore performed a series of experiments on the direct stimulation of the muscle arteries with the following results.

In order to confine the stimulation to the small artery under observation, and at the same time not to injure the vessel or the muscle by the application of the electrodes, I attached to each of the platinum points a long, thin, very finely pointed strip of tinfoil, and arranged so that I could place each tinfoil point so as just to touch the muscle at any particular spot I desired. In order to use the same magnifying power as in my other experiments, I at first placed these electrodes underneath the muscle and moved them upwards, until I could see on looking through the microscope that their points just touched the muscle at the right places; finding, however, that it was very difficult to be sure of placing them rightly in position in this way, I discarded the higher object-lens, and in most of my observations made use of a power, by which every space of the micrometer scale corresponded to $\frac{1}{80}$th m.m. With this power I was able to insert the tinfoil strips between the lens and the muscle, and could therefore easily manipulate, so that the points alone of the tinfoil strips touched the muscle wherever I pleased. Again seeing that, except with a very weak current, the contraction of the muscular fibres between the electrodes is necessarily so strong, as to prevent at all accurate measurement of the artery under observation during the stimulation itself, I adopted the plan of limiting the time of stimulation to one second or less, and to prevent the possibility of the tinfoil points holding the muscle in its contracted position, I lifted them rapidly from the muscle at the end of the stimulation; by this means I was able to measure accurately the calibre of the artery in less than ten seconds after the commencement of the stimulation, a time sufficiently short, so it seems to me, to make certain, whether the primary effect of the stimulation was a constriction or a dilata-

tion of the vessel. By these methods I found that, whether the tinfoil points were placed so that the current passed longitudinally along the axis of the artery, or transversely across that axis, with a stimulus so weak as to cause a contraction of the muscle not sufficient to interfere with the measurement of the artery, the vessel was seen to dilate steadily from the commencement of the stimulation, without any sign of previous constriction, the dilatation occurring during the stimulation itself and continuing after it was over, if the time of stimulation was short enough; in fact, the dilatation produced was quite similar to that caused by stimulation of the nerve; in the case of a stronger stimulation lasting less than one second, the artery was found to be dilated as soon as it could be measured again, and this dilatation continued until it had reached its maximum about 30″ after the stimulation, the maximum being as great as could be produced by stimulation of the nerve; upon still further increasing the strength of the stimulation, a point was at last reached, when instead of an increasing dilatation of the vessel after a stimulation of less than one second duration, the artery under observation was found to be most markedly constricted as soon as the measurement was again possible, and this constriction so caused was by no means temporary, but instead lasted for much the same length of time after the stimulation, as the constriction caused in the arteries of the web by the same mode of procedure, the vessel remaining nearly absolutely closed for some time and difficult to see, and then gradually reopening, until at last it reached a size decidedly greater than before the stimulation, although not so great as was previously obtained by using a slightly weaker strength of current. The dilatation that follows upon a weak or moderately strong stimulation occurs whether the nerve has been previously cut or not. Frequently when the electrodes have been placed transversely, so that the current crosses the artery, it is seen, that the dilatation caused by stimulation is confined or nearly confined to that particular part of the vessel between the electrodes, a most marked bulging taking place at this spot, the rest of the artery and the neighbouring vessels being hardly if at all altered; and then, upon stimulating with a much stronger current, this part of the vessel alone is constricted

to nearly absolute closure, the rest of the artery both in front of and behind the affected piece being somewhat dilated, with a more rapid full stream on the proximal side and a full sluggish stream on the distal side; this local constriction being followed by a decided local dilatation of the same part. The arteries some little distance removed from the electrodes are quite unaffected, remaining of the same calibre throughout. I am unable as yet to say in what way the change takes place from dilatation on stimulation to constriction on stimulation, although, from what I have noticed in one case, the sequence of events seemed rather to be, that up to a certain strength of current dilatation alone was observed, then with a very slightly stronger current a brief constriction quickly followed by dilatation took place, and with the increase of the strength of the current the constriction became more lasting and more manifest, the subsequent dilatation occurring later, until a strength was reached, which caused a long-enduring very marked constriction of the vessel.

From these experiments two things appear to me clear, 1st, that direct stimulation of an artery does not necessarily cause that artery to contract, but may cause it to dilate; 2nd, that the unstriped muscular fibres of the arteries of muscle are not very easily exhausted; for, if this were the case, one would expect to find constriction of those vessels most marked upon weak stimulation, and dilatation of them upon strong stimulation, whereas the reverse is the case.

Seeing then, that in the case of the arteries of this muscle it is possible to cause dilatation by nerve stimulation, after the circulation through the muscle has been removed, and also, that direct excitation of the artery causes it to dilate if the stimulation is not too strong, and to contract if it is very strong, one seems driven to seek for some other explanation of vaso-dilator action than the ordinarily accepted theory of inhibition; if, for instance, one could assume, that an unstriped muscular fibre possesses the power of contracting in two directions, longitudinally and transversely, so that in the one case the fibre becomes thicker and shorter, and in the other thinner and longer, then the difference between vaso-constrictor and vaso-dilator nerve-fibres would consist in the assumption, that in the first case the

nerve-fibre terminates in the muscle in such a way, as to cause upon its excitation a contraction in the direction of the long axis, and in the second case a contraction in the direction of one of the short axes of the muscle-fibre.

However, upon the supposition, that dilatation of an artery is caused not by the removal of contraction but by contraction in another direction, it is reasonable to assume that, just as it is possible to cause constriction of arteries by nerve stimulation in a tissue that has been removed from the body, so it should be possible to cause dilatation of the arteries of the mylohyoid muscle by stimulation of its nerve, when the muscle has been entirely removed from the body. In all my attempts to obtain dilatation in this way I have completely failed; stimulation of the nerve of the isolated curarised muscle, which has been separately pinned out under the microscope, producing no effect whatever upon the size of the muscular arteries. I am inclined therefore at present to imagine, that it is not possible to produce dilatation of an artery from which all internal pressure has been removed, and that in the cases where dilatation was caused after removal of the heart, there was really sufficient blood-pressure left in the whole vascular system to cause the amount of dilatation observed upon the hypothesis of inhibitory action alone. One must then explain the dilatation caused by direct stimulation of an artery as due to excitation of the endings of the vaso-dilator nerves, while the marked constriction that occurs upon very strong direct stimulation would be due to the excitation of the circular muscle fibres themselves overpowering the action of the dilator fibres. Although therefore for the present I do not deem the evidence strong enough to overthrow the accepted theory of inhibitory action, yet it might possibly be worth while to investigate, whether between the simple unspecialized contractile protoplasm of the amœba, capable of contraction in all directions, and the highly specialized striated muscle fibre, intermediate forms of contractile tissue may not be found, in which the power of contraction is limited along certain axes; and whether the smooth unstriped muscular fibre may not represent one of these intermediate stages.

The title borne by the preceding pages should perhaps have been "Studies from the Biological Institute," since all the investigations described were not carried on in the Physiological Laboratory. The genetic connection however between this and the preceding parts rendered it desirable to retain the old title.

Pl. IV.

Fig. 1. Fig. 2. Fig. 3. Fig. 4. Fig. 5. Fig. 6.

J. F. Bullar, delt. T. P. Collings.

Plate V.

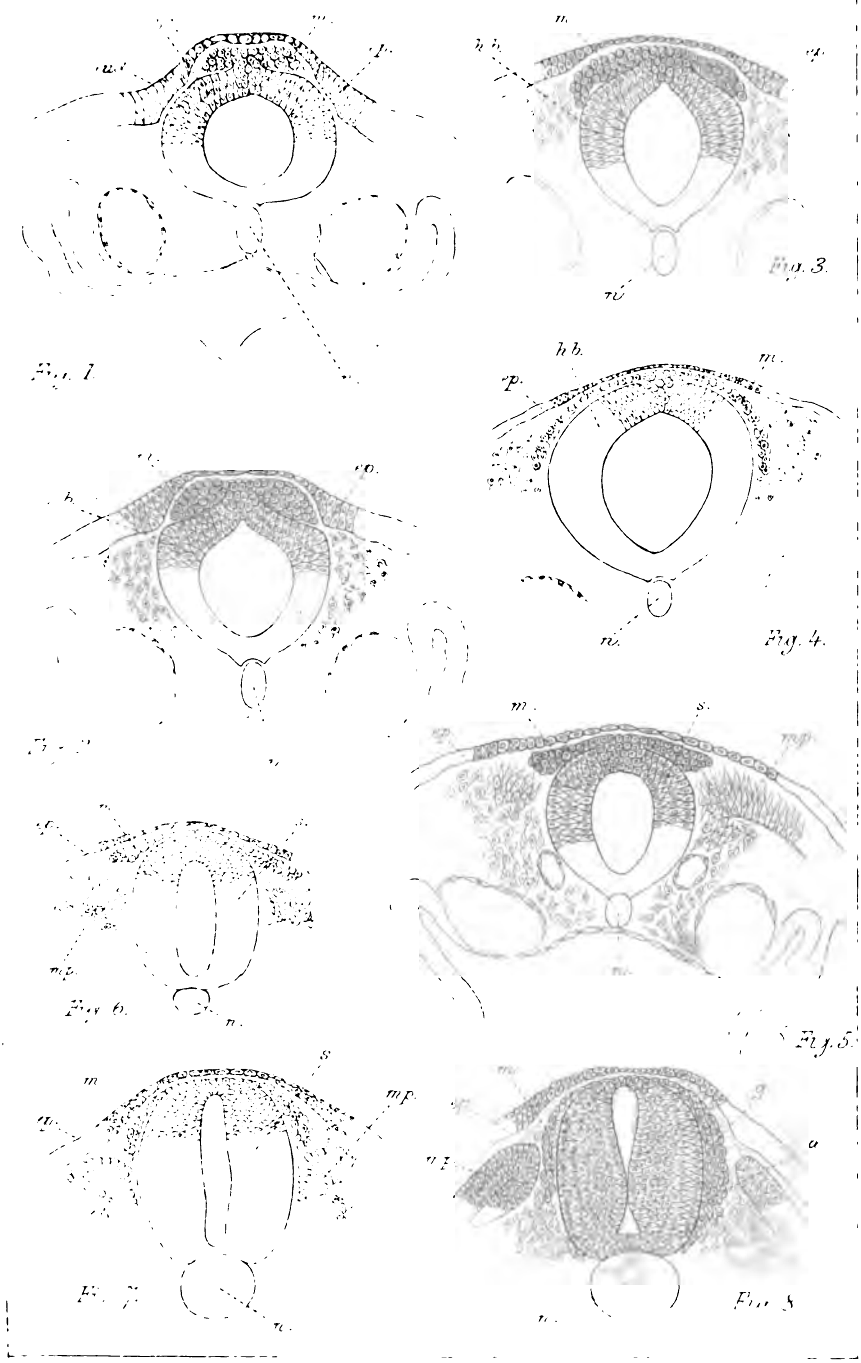
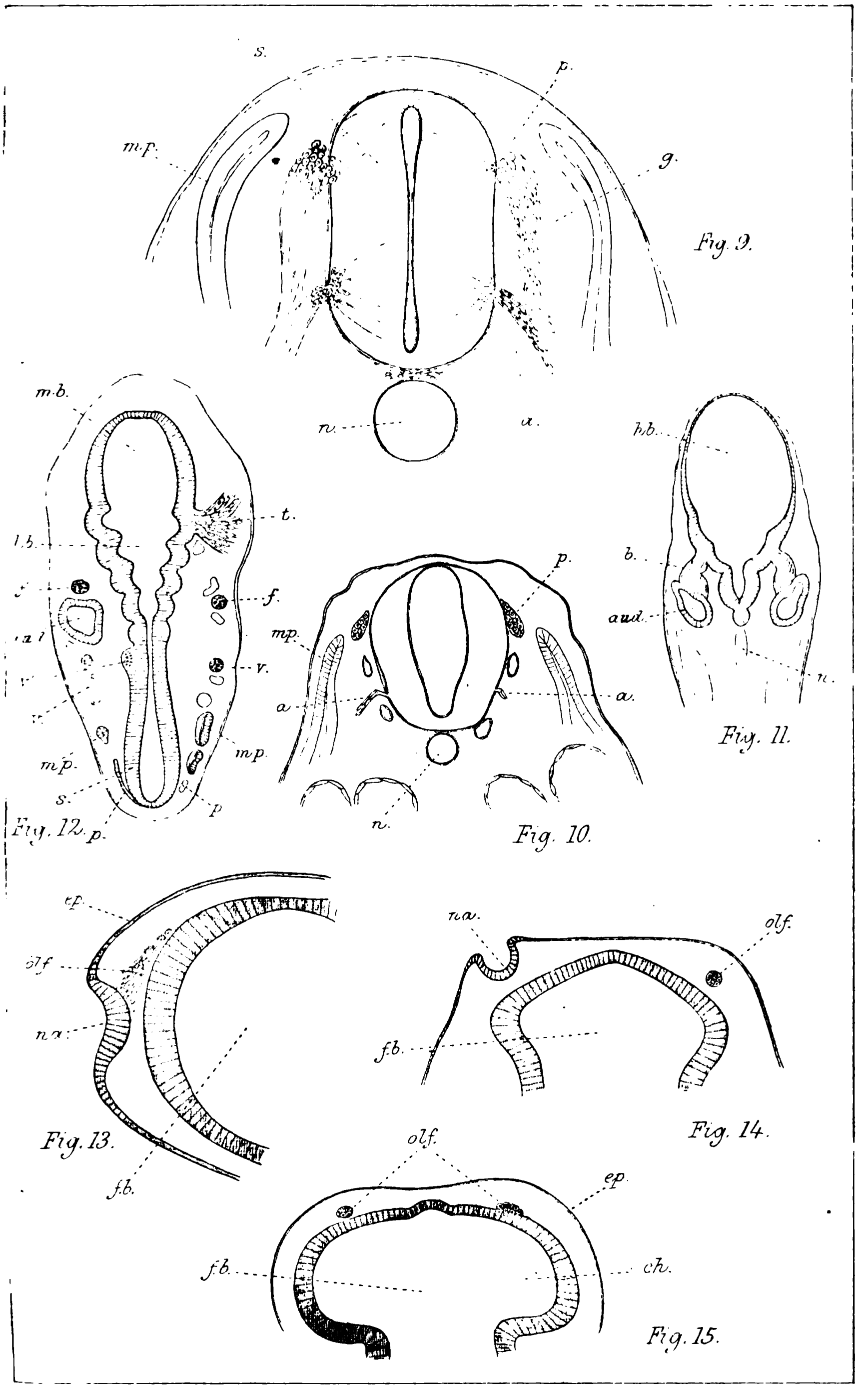
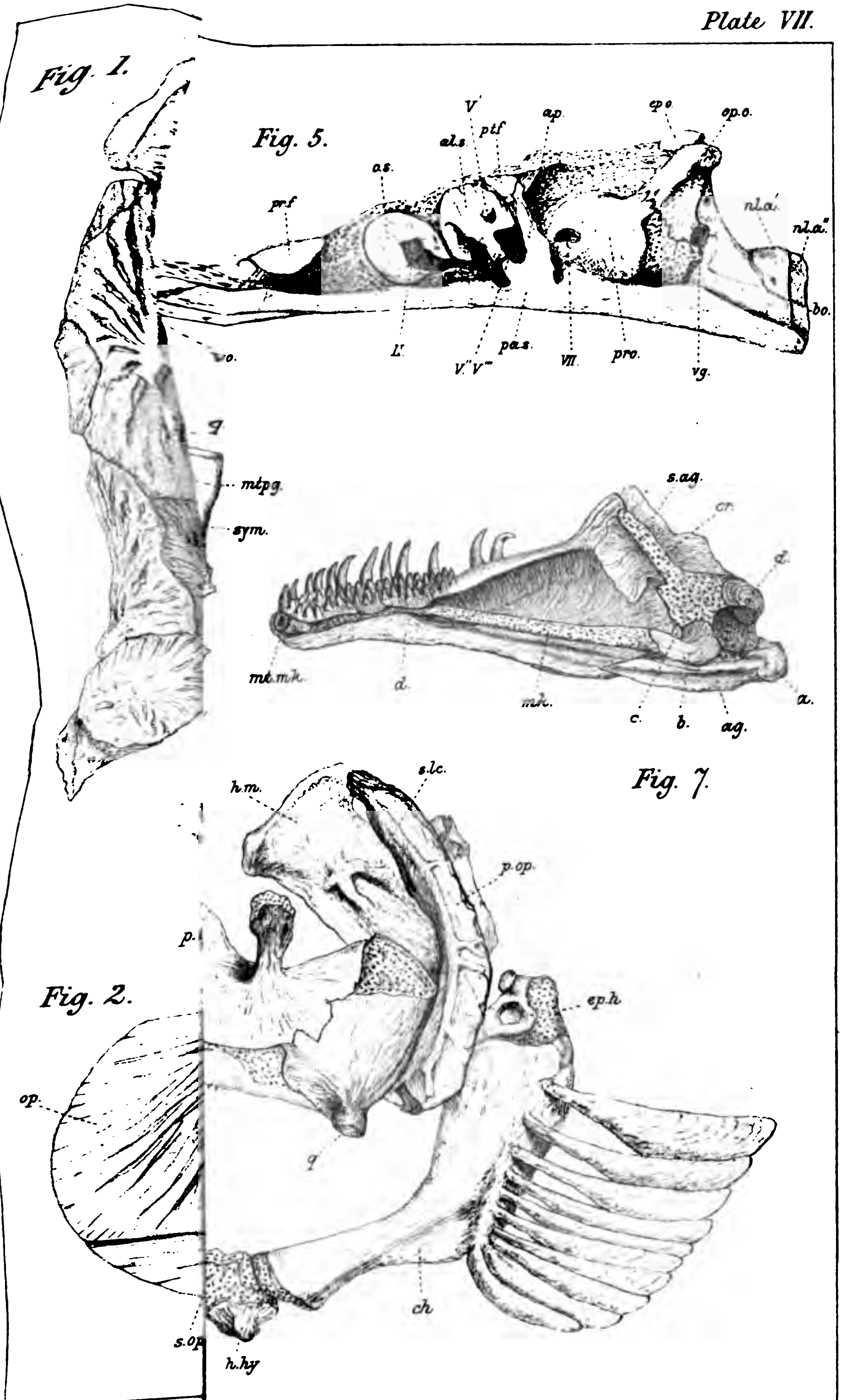


Plate VI.



A. M. Marshall del.t F. Huth, Lith.r Edin.r

Plate VII.



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